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STISTANT COMMISSIONER OF PATENTS AND TRADEMARKS shington DC 20231

Transmitted herewith for filing is the patent application of Inventors: Heinrich D. LÜTTICKEN, Egbert MUNDT and Adriaan A.W.M. LOON

For: RECOMBINANT BIRNAVIRUS VACCINE

[X] Specification and claims (61 pages)

[X] Five (5) sheets of drawings.

[] An assignment of the invention to

[X] Sequence Listing (Paper and CRF Disk)

[X] Preliminary Amendment

[X] Information Disclosure Statement/PTO Form 1449/References

[X] A filing fee calculated as shown below:

	FOR:		NO.	FILED	NO.	EXTRA		RATE			FEE
	BASIC I	FEE								\$ 7	790.00
	TOTAL C	CLAIMS	29-	20 =		9	X	\$ 22		\$:	L98.00
,40 2	INDEP C	CLAIMS		3 =		0	X	\$ 82		\$_	.00
	[X] MUI	LTIPLE	DEPENDENT	CLAIMS	PRESENTE)	+	\$270		\$ 2	270.00
						 		TOT	TAL	\$1:	258.00
						No. <u>02-2334</u> ssignment r			of	\$12	258.00

The Commissioner is hereby authorized to charge payment for [X] following fees associated with this communication or credit overpayment to Deposit Account No. 02-2334.

[X] Any additional filing fees required under 37 CFR 1.16.

[X] The Commissioner is hereby authorized to charge payment for the following fees associated with this communication or credit overpayment to Deposit Account No. 02-2334.

[X] Any patent application processing fees under 37 CFR 1.17.

[X] Any filing fees under 37 CFR 1.16 for presentation of extra claims.

> Respectfully submitted, AKZO NOBEL N.V.

Mary E. Gormley By:

> Attorney for Applicants Registration No. 34,409

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Enclosures

Attorney Docket No. I/97269 US

Express Mail No. EL042439813US

58lutckn.fil

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Heinrich D. LÜTTICKEN, Egbert MUNDT and Adriaan A.W.M. LOON

Serial Number: to be assigned Group Art Unit: to be assigned

Filed: Concurrently herewith Examiner: to be assigned

For: RECOMBINANT BIRNAVIRUS VACCINE

PRELIMINARY AMENDMENT AND SUBMISSION OF SEQUENCE LISTING

Assistant Commissioner of Patents Washington, D.C. 20231

May 26, 1998

Sir:

Prior to the calculation of the fee in the above-identified application, please make the following amendments:

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 1, above line 4, insert -- Field of the Invention --; and between lines 6 and 7, insert -- Background of the Invention --.

Page 3, line 14, insert -- <u>Summary of the Invention</u> --; and line 21, insert -- <u>Detailed Description of the</u>
Invention --.

Please delete pages 29 - 58 in their entireties and replace them with the attached Sequence Listing as pages 29 - 56.

Please renumber pages 59 - 61 as pages 57 - 59, respectively.

Express Mail No. EL042439813US

IN THE CLAIMS:

Please amend the claims as follows:

- 2. (amended) A birnavirus mutant according to claim 1, [characterised in that] wherein the mutation is a substitution.
- 3. (amended) A birnavirus mutant according to claim 1, [characterised in that] wherein the mutation is an insertion of a heterologous nucleic acid sequence.
- 4. (amended) A birnavirus mutant according to claim 3, [characterised in that] wherein the heterologous nucleic acid sequence encodes a polypeptide and the heterologous nucleic acid sequence is under the control of an expression control sequence regulating the expression of the sequence in a cell infected with the virus mutant.
- 5. (amended) A birnavirus mutant according to [claims 1-4, characterised in that] claim 1, wherein the birnavirus is infectious bursal disease virus (IBDV).
- 6. (amended) A birnavirus mutant according to claim 5, [characterised in that] wherein the mutation is in the genome of a virulent field virus.
- 7. (amended) A birnavirus mutant according to claim 5, [characterised in that] wherein the mutation is in the genome of a vaccine strain[, preferably in vaccine strain D78].
- 8. (amended) A birnavirus mutant according to [claims 5-7, characterised in that] <u>claim 5, wherein</u> the mutant has a mutated start codon and three stop codons in the 5'-end of the VP5 gene as shown in SEQ ID NO:7.

- 9. (amended) A birnavirus according to [claims 5-8, characterised in that] <u>claim 5, wherein</u> the IBDV expresses a chimeric VP2 protein comprising virus neutralizing epitopes of different antiqenic IBDV types.
- 10. (amended) A vaccine against a birnavirus infection in animals, [characterised in that it comprises] comprising a birnavirus mutant according to any one of claims 1-9 and a pharmaceutically acceptable carrier.

Please cancel claim 11 without prejudice or disclaimer of the subject matter thereof.

- 12. (amended) A method [according to clam 11, characterised in that the method comprises] for determining birnavirus infection in an animal, comprising the steps of:
- (i) incubating a sample suspected of containing antibirnavirus antibodies[,] with VP5 antigen,
- (ii) allowing the formation of antibody-antigen complex, and
- (iii) detecting the presence of the antibody-antigen complex,

wherein the presence of the complex indicates a birnavirus infection.

13. (amended) A diagnostic kit suitable for carrying out a method according to [claims 11-12] claim 12, comprising VP5 antigen coated on a solid phase.

Please cancel claim 14 without prejudice or disclaimer of the subject matter thereof.

Please add the following new claims 15 - 31.

- -- 15. A birnavirus mutant according to claim 7, wherein the vaccine strain is D78. --
- -- 16. A diagnostic test kit according to claim 13, further comprising an enzyme-conjugated antibody and substrate to said enzyme. --
- -- 17. A method for determining birnavirus infection in an animal, comprising:
- (i) incubating a sample suspected of containing VP5 with anti-birnavirus VP5 antibody;
 - (ii) allowing the formation of antibody-antigen complex; and
- (iii) detecting the presence of antibody-antigen complex, wherein the presence of the complex indicates birnavirus infection. --
- -- 18. A diagnostic test kit for carrying out a method according to claim 17, comprising a container having anti-birnavirus VP5 antibody. --
- -- 19. A diagnostic test kit according to claim 18, further comprising a second labelled antibody which will detect said complex. --
- -- 20. A diagnostic test kit according to claim 18, wherein the antibody is labelled. --
- -- 21. A diagnostic test kit according to claim 18, wherein the antibody is coated on a solid phase. --
- -- 22. A birnavirus according to claim 2, wherein the birnavirus is IBDV. --

- -- 23. A birnavirus according to claim 3, wherein the birnavirus is IBDV. --
- -- 24. A birnavirus according to claim 22, wherein the mutation is in the genome of a virulent field virus. --
- -- 25. A birnavirus according to claim 23, wherein the mutation is in the genome of a virulent field virus. --
- -- 26. A birnavirus according to claim 22, wherein the mutation is in the genome of a vaccine strain. --
- -- 27. A birnavirus according to claim 23, wherein the mutation is in the genome of a vaccine strain. --
- -- 28. A birnavirus according to claim 26, wherein the vaccine strain is D78. --
- -- 29. A birnavirus according to claim 27, wherein the vaccine strain is D78. --
- -- 30. A birnavirus according to claim 6, wherein the IBDV expresses a chimeric VP2 protein comprising virus neutralizing epitopes of different antigenic IBDV types. --
- -- 31. A vaccine against a birnavirus infection in animals, comprising a birnavirus mutant according to any one of claims 22 30 and a pharmaceutically acceptable carrier. --

REMARKS

Claims 2 - 10, 12 and 13 are amended, claims 11 and 14 canceled, and claims 15 - 31 are added, hereby. Claims 1 - 10, 12, 13 and 15 - 31 are presented for examination.

Also submitted herewith is the Sequence Listing in both paper and CRF diskette. The name of the file on the diskette is 58LUTTIC.SEQ. The paper copy and CRF are the same and the sequences thereof are the same as in the original specification. No new matter has been added.

It is believed that claims 1 - 10, 12, 13 and 15 - 31 recite a patentable improvement in the art. Favorable action is solicited. In the event any fees are required with this paper, please charge our Deposit Account No. 02-2334.

Respectfully submitted,

Mary E. Gormley

Attorney for Applicants Registration No. 34,409

Attorney Docket I/97269 US

AKZO NOBEL N.V. 1300 Piccard Drive, Suite 206 Rockville, Maryland 20850-4373

Tel: (301) 948-7400 Fax: (301) 948-9751

MEG:ms

Enclosure: Sequence Listing (Paper Copy and CRF)

58lutckn.pre

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
 - (i) APPLICANT: Lutticken, Heinrich D. Mundt, Egbert Loon, Adriaan A. W. M.
 - (ii) TITLE OF INVENTION: Recombinant birnavirus vaccine
 - (iii) NUMBER OF SEQUENCES: 8
 - (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Akzo Nobel Patent Dept.
 - (B) STREET: 1300 Piccard Drive, Suite 206
 - (C) CITY: Rockville
 - (D) STATE: Maryland
 - (E) COUNTRY: US
 - (F) ZIP: 20850
 - (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30(EPO)
 - (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE: 26-MAY-1998
 - (C) CLASSIFICATION:
 - (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Gormley, Mary E.
 - (B) REGISTRATION NUMBER: 34,409
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 301-948-7400
 - (B) TELEFAX: 301-948-9751
- (2) INFORMATION FOR SEQ ID NO: 1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2827 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 112..2745

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGATACGATG (GGATACGATG GGTCTGACCC TCTGGGAGTC ACGAATTAAC GTGGCTACTA GGGGCGATAC 60													
CCGCCGCTGG	CCGCCACGTT AG	STGGCTCCT CTT	CTTGATG ATT	CTGCCAC C ATO Met 1	Ser									
GAC ATT TTC Asp Ile Phe 5	AAC AGT CCA Asn Ser Pro	CAG GCG CGA 2 Gln Ala Arg 3 10	AGC ACG ATC Ser Thr Ile	C TCA GCA GCG Ser Ala Ala 15	TTC 165 Phe									
GC ATA AAG Gly Ile Lys C 20	CCT ACT GCT Pro Thr Ala	GGA CAA GAC G Gly Gln Asp 1 25	GTG GAA GAA Val Glu Glu 30	CTC TTG ATC Leu Leu Ile	CCT 213 Pro									
AAA GTT TGG Lys Val Trp	GTG CCA CCT Val Pro Pro 40	GAG GAT CCG (Glu Asp Pro	CTT GCC AGC Leu Ala Ser 45	CCT AGT CGA Pro Ser Arg	CTG 261 Leu 50									
GCA AAG TTC Ala Lys Phe	CTC AGA GAG Leu Arg Glu 55	AAC GGC TAC AASn Gly Tyr	AAA GTT TTG Lys Val Leu 60	CAG CCA CGG Gln Pro Arg 65	TCT 309 Ser									
ETG CCC GAG Leu Pro Glu	AAT GAG GAG Asn Glu Glu 70	TAT GAG ACC Tyr Glu Thr 75	GAC CAA ATA Asp Gln Ile	CTC CCA GAC Leu Pro Asp 80	TTA 357 Leu									
GCA TGG ATG Ala Trp Met 85	CGA CAG ATA Arg Gln Ile	GAA GGG GCT (Glu Gly Ala ' 90	GTT TTA AAA Val Leu Lys	CCC ACT CTA Pro Thr Leu 95	TCT 405 Ser									
CTC CCT ATT Leu Pro Ile 100	GGA GAT CAG Gly Asp Gln	GAG TAC TTC (Glu Tyr Phe 1	CCA AAG TAC Pro Lys Tyr 110	TAC CCA ACA Tyr Pro Thr	CAT 453 His									
CGC CCT AGC Arg Pro Ser 115	AAG GAG AAG Lys Glu Lys 120	CCC AAT GCG ! Pro Asn Ala !	TAC CCG CCA Tyr Pro Pro 125	GAC ATC GCA Asp Ile Ala	CTA 501 Leu 130									
CTC AAG CAG Leu Lys Gln	ATG ATT TAC Met Ile Tyr 135	Leu Phe Leu (CAG GTT CCA Gln Val Pro 140	GAG GCC AAC Glu Ala Asn 145	GAG 549 Glu									

						ACC Thr										597
						TAC Tyr										645
ATG Met	AAG Lys 180	GAG Glu	GTC Val	GCC Ala	ACT Thr	GGA Gly 185	AGA Arg	AAC Asn	CCA Pro	AAC Asn	AAG Lys 190	GAT Asp	CCT Pro	CTA Leu	AAG Lys	693
CTT Leu 195	GGG Gly	TAC Tyr	ACT Thr	TTT Phe	GAG Glu 200	AGC Ser	ATC Ile	GCG Ala	CAG Gln	CTA Leu 205	CTT Leu	GAC Asp	ATC Ile	ACA Thr	CTA Leu 210	741
CCG Pro	GTA Val	GGC Gly	CCA Pro	CCC Pro 215	GGT Gly	GAG Glu	GAT Asp	GAC Asp	AAG Lys 220	CCC Pro	TGG Trp	GTG Val	CCA Pro	CTC Leu 225	ACA Thr	789
AGA Arg	GTG Val	CCG Pro	TCA Ser 230	CGG Arg	ATG Met	TTG Leu	GTG Val	CTG Leu 235	ACG Thr	GGA Gly	GAC Asp	GTA Val	GAT Asp 240	GGC Gly	GAC Asp	837
TTT Phe	GAG Glu	GTT Val 245	GAA Glu	GAT Asp	TAC Tyr	CTT Leu	CCC Pro 250	AAA Lys	ATC Ile	AAC Asn	CTC Leu	AAG Lys 255	TCA Ser	TCA Ser	AGT Ser	885
GGA Gly	CTA Leu 260	CCA Pro	TAT Tyr	GTA Val	GGT Gly	CGC Arg 265	ACC Thr	AAA Lys	GGA Gly	GAG Glu	ACA Thr 270	ATT Ile	GGC Gly	GAG Glu	ATG Met	933
ATA Ile 275	GCT Ala	ATC Ile	TCA Ser	AAC Asn	CAG Gln 280	TTT Phe	CTC Leu	AGA Arg	GAG Glu	CTA Leu 285	TCA Ser	ACA Thr	CTG Leu	TTG Leu	AAG Lys 290	981
CAA Gln	GGT Gly	GCA Ala	GGG Gly	ACA Thr 295	AAG Lys	GGG Gly	TCA Ser	AAC Asn	AAG Lys 300	AAG Lys	AAG Lys	CTA Leu	CTC Leu	AGC Ser 305	ATG Met	1029
TTA Leu	AGT Ser	GAC Asp	TAT Tyr 310	TGG Trp	TAC Tyr	TTA Leu	TCA Ser	TGC Cys 315	GGG Gly	CTT Leu	TTG Leu	TTT Phe	CCA Pro 320	AAG Lys	GCT Ala	1077
GAA Glu	AGG Arg	TAC Tyr 325	GAC Asp	AAA Lys	AGT Ser	ACA Thr	TGG Trp 330	CTC Leu	ACC Thr	AAG Lys	ACC Thr	CGG Arg 335	AAC Asn	ATA Ile	TGG Trp	1125
TCA Ser	GCT Ala 340	CCA Pro	TCC Ser	CCA Pro	ACA Thr	CAC His 345	CTC Leu	ATG Met	ATC Ile	TCT Ser	ATG Met 350	ATC Ile	ACC Thr	TGG Trp	CCC Pro	1173

	ATG Met															;	1221
	CTC Leu																1269
	TGG Trp															:	1317
AAC Asn	ATA Ile	TAC Tyr 405	ATT Ile	GTC Val	CAC His	TCA Ser	AAC Asn 410	ACG Thr	TGG Trp	TAC Tyr	TCA Ser	ATT Ile 415	GAC Asp	CTA Leu	GAG Glu	:	1365
AAG Lys	GGT Gly 420	GAG Glu	GCA Ala	AAC Asn	TGC Cys	ACT Thr 425	CGC Arg	CAA Gln	CAC His	ATG Met	CAA Gln 430	GCC Ala	GCA Ala	ATG Met	TAC Tyr	:	1413
TAC Tyr 435	ATA Ile	CTC Leu	ACC Thr	AGA Arg	GGG Gly 440	TGG Trp	TCA Ser	GAC Asp	AAC Asn	GGC Gly 445	GAC Asp	CCA Pro	ATG Met	TTC Phe	AAT Asn 450	:	1461
CAA Cln	ACA Thr	TGG Trp	GCC Ala	ACC Thr 455	TTT Phe	GCC Ala	ATG Met	AAC Asn	ATT Ile 460	GCC Ala	CCT Pro	GCT Ala	CTA Leu	GTG Val 465	GTG Val		1509
GAC Asp	TCA Ser	TCG Ser	TGC Cys 470	CTG Leu	ATA Ile	ATG Met	AAC Asn	CTG Leu 475	CAA Gln	ATT Ile	AAG Lys	ACC Thr	TAT Tyr 480	GGT Gly	CAA Gln	;	1557
GGC Gly	AGC Ser	GGG Gly 485	AAT Asn	GCA Ala	GCC Ala	ACG Thr	TTC Phe 490	ATC Ile	AAC Asn	AAC Asn	CAC His	CTC Leu 495	TTG Leu	AGC Ser	ACA Thr	:	1605
CTA Leu	GTG Val 500	CTT Leu	GAC Asp	CAG Gln	TGG Trp	AAC Asn 505	CTG Leu	ATG Met	AGA Arg	CAG Gln	CCC Pro 510	AGA Arg	CCA Pro	GAC Asp	AGC Ser	:	1653
GAG Glu 515	GAG Glu	TTC Phe	AAA Lys	TCA Ser	ATT Ile 520	GAG Glu	GAC Asp	AAG Lys	CTA Leu	GGT Gly 525	ATC Ile	AAC Asn	TTT Phe	AAG Lys	ATT Ile 530	:	1701
GAG Glu	AGG Arg	TCC Ser	ATT Ile	GAT Asp 535	GAT Asp	ATC Ile	AGG Arg	GGC Gly	AAG Lys 540	CTG Leu	AGA Arg	CAG Gln	CTT Leu	GTC Val 545	CTC Leu	:	1749
CTT Leu	GCA Ala	CAA Gln	CCA Pro 550	GGG Gly	TAC Tyr	CTG Leu	AGT Ser	GGG Gly 555	GGG Gly	GTT Val	GAA Glu	CCA Pro	GAA Glu 560	CAA Gln	TCC Ser	:	1797

													ACA Thr			1845
													CGC Arg			1893
													CTC Leu			1941
AAA Lys	GTC Val	GGG Gly	ATC Ile	GAG Glu 615	CAG Gln	GCA Ala	TAC Tyr	AAG Lys	GTA Val 620	GTC Val	AGG Arg	TAT Tyr	GAG Glu	GCG Ala 625	TTG Leu	1989
AGG Arg	TTG Leu	GTA Val	GGT Gly 630	GGT Gly	TGG Trp	AAC Asn	TAC Tyr	CCA Pro 635	CTC Leu	CTG Leu	AAC Asn	AAA Lys	GCC Ala 640	TGC Cys	AAG Lys	2037
AAT Asn	AAC Asn	GCA Ala 645	GGC Gly	GCC Ala	GCT Ala	CGG Arg	CGG Arg 650	CAT His	CTG Leu	GAG Glu	GCC Ala	AAG Lys 655	GGG Gly	TTC Phe	CCA Pro	2085
CTC Leu	GAC Asp 660	GAG Glu	TTC Phe	CTA Leu	GCC Ala	GAG Glu 665	TGG Trp	TCT Ser	GAG Glu	CTG Leu	TCA Ser 670	GAG Glu	TTC Phe	GGT Gly	GAG Glu	2133
GCC Ala 675	TTC Phe	GAA Glu	GGC Gly	TTC Phe	AAT Asn 680	ATC Ile	AAG Lys	CTG Leu	ACC Thr	GTA Val 685	ACA Thr	TCT Ser	GAG Glu	AGC Ser	CTA Leu 690	2181
GCC Ala	GAA Glu	CTG Leu	AAC Asn	AAG Lys 695	CCA Pro	GTA Val	CCC Pro	CCC Pro	AAG Lys 700	CCC Pro	CCA Pro	AAT Asn	GTC Val	AAC Asn 705	AGA Arg	2229
CCA Pro	GTC Val	AAC Asn	ACT Thr 710	GGG Gly	GGA Gly	CTC Leu	AAG Lys	GCA Ala 715	GTC Val	AGC Ser	AAC Asn	GCC Ala	CTC Leu 720	AAG Lys	ACC Thr	2277
GGT Gly	CGG Arg	TAC Tyr 725	AGG Arg	AAC Asn	GAA Glu	GCC Ala	GGA Gly 730	CTG Leu	AGT Ser	GGT Gly	CTC Leu	GTC Val 735	CTT Leu	CTA Leu	GCC Ala	2325
ACA Thr	GCA Ala 740	AGA Arg	AGC Ser	CGT Arg	CTG Leu	CAA Gln 745	GAT Asp	GCA Ala	GTT Val	AAG Lys	GCC Ala 750	AAG Lys	GCA Ala	GAA Glu	GCC Ala	2373
GAG Glu 755	AAA Lys	CTC Leu	CAC His	AAG Lys	TCC Ser 760	AAG Lys	CCA Pro	GAC Asp	GAC Asp	CCC Pro 765	GAT Asp	GCA Ala	GAC Asp	TGG Trp	TTC Phe 770	2421

GAA Glu	AGA Arg	TCA Ser	GAA Glu	ACT Thr 775	CTG Leu	TCA Ser	GAC Asp	CTT Leu	CTG Leu 780	GAG Glu	AAA Lys	GCC Ala	GAC Asp	ATC Ile 785	GCC Ala	2469
AGC Ser	AAG Lys	GTC Val	GCC Ala 790	CAC His	TCA Ser	GCA Ala	CTC Leu	GTG Val 795	GAA Glu	ACA Thr	AGC Ser	GAC Asp	GCC Ala 800	CTT Leu	GAA Glu	2517
GCA Ala	GTT Val	CAG Gln 805	TCG Ser	ACT Thr	TCC Ser	GTG Val	TAC Tyr 810	ACC Thr	CCC Pro	AAG Lys	TAC Tyr	CCA Pro 815	GAA Glu	GTC Val	AAG Lys	2565
AAC Asn	CCA Pro 820	CAG Gln	ACC Thr	GCC Ala	TCC Ser	AAC Asn 825	CCC Pro	GTT Val	GTT Val	GGG Gly	CTC Leu 830	CAC His	CTG Leu	CCC Pro	GCC Ala	2613
AAG Lys 835	AGA Arg	GCC Ala	ACC Thr	GGT Gly	GTC Val 840	CAG Gln	GCC Ala	GCT Ala	CTT Leu	CTC Leu 845	GGA Gly	GCA Ala	GGA Gly	ACG Thr	AGC Ser 850	2661
AGA Arg	CCA Pro	ATG Met	GGG Gly	ATG Met 855	GAG Glu	GCC Ala	CCA Pro	ACA Thr	CGG Arg 860	TCC Ser	AAG Lys	AAC Asn	GCC Ala	GTG Val 865	AAA Lys	2709
ATG Met	GCC Ala	AAA Lys	CGG Arg 870	CGG Arg	CAA Gln	CGC Arg	CAA Gln	AAG Lys 875	GAG Glu	AGC Ser	CGC Arg	TAAC	CAGC	CAT		2755
GATO	GGAI	ACC I	ACTC	AAGA <i>I</i>	AG AC	GAC	CTA	A TCC	CCAGA	CCC	CGT	ATCC	CCG (CCTI	CGCCT	2815
ECGO	GGGG	ecc o	cc													2827

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 878 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ser Asp Ile Phe Asn Ser Pro Gln Ala Arg Ser Thr Ile Ser Ala 1 5 10 15

Ala Phe Gly Ile Lys Pro Thr Ala Gly Gln Asp Val Glu Glu Leu Leu 20 25 30

Ile Pro Lys Val Trp Val Pro Pro Glu Asp Pro Leu Ala Ser Pro Ser 35 40 45

Arg Leu Ala Lys Phe Leu Arg Glu Asn Gly Tyr Lys Val Leu Gln Pro Arg Ser Leu Pro Glu Asn Glu Glu Tyr Glu Thr Asp Gln Ile Leu Pro Asp Leu Ala Trp Met Arg Gln Ile Glu Gly Ala Val Leu Lys Pro Thr Leu Ser Leu Pro Ile Gly Asp Gln Glu Tyr Phe Pro Lys Tyr Tyr Pro Thr His Arg Pro Ser Lys Glu Lys Pro Asn Ala Tyr Pro Pro Asp Ile 115 120 Ala Leu Leu Lys Gln Met Ile Tyr Leu Phe Leu Gln Val Pro Glu Ala 130 135 Asn Glu Gly Leu Lys Asp Glu Val Thr Leu Leu Thr Gln Asn Ile Arg 155 Asp Lys Ala Tyr Gly Ser Gly Thr Tyr Met Gly Gln Ala Asn Arg Leu Wal Ala Met Lys Glu Val Ala Thr Gly Arg Asn Pro Asn Lys Asp Pro 185 Leu Lys Leu Gly Tyr Thr Phe Glu Ser Ile Ala Gln Leu Leu Asp Ile 200 Thr Leu Pro Val Gly Pro Pro Gly Glu Asp Asp Lys Pro Trp Val Pro Leu Thr Arg Val Pro Ser Arg Met Leu Val Leu Thr Gly Asp Val Asp 225 230 240 Gly Asp Phe Glu Val Glu Asp Tyr Leu Pro Lys Ile Asn Leu Lys Ser 250 255 Ser Ser Gly Leu Pro Tyr Val Gly Arg Thr Lys Gly Glu Thr Ile Gly Glu Met Ile Ala Ile Ser Asn Gln Phe Leu Arg Glu Leu Ser Thr Leu 275 280 285 Leu Lys Gln Gly Ala Gly Thr Lys Gly Ser Asn Lys Lys Leu Leu 290 295 Ser Met Leu Ser Asp Tyr Trp Tyr Leu Ser Cys Gly Leu Leu Phe Pro 310 315

Lys Ala Glu Arg Tyr Asp Lys Ser Thr Trp Leu Thr Lys Thr Arg Asn Ile Trp Ser Ala Pro Ser Pro Thr His Leu Met Ile Ser Met Ile Thr Trp Pro Val Met Ser Asn Ser Pro Asn Asn Val Leu Asn Ile Glu Gly Cys Pro Ser Leu Tyr Lys Phe Asn Pro Phe Arg Gly Gly Leu Asn Arg Ile Val Glu Trp Ile Leu Ala Pro Glu Glu Pro Lys Ala Leu Val Tyr Ala Asp Asn Ile Tyr Ile Val His Ser Asn Thr Trp Tyr Ser Ile Asp Leu Glu Lys Gly Glu Ala Asn Cys Thr Arg Gln His Met Gln Ala Ala Met Tyr Tyr Ile Leu Thr Arg Gly Trp Ser Asp Asn Gly Asp Pro Met Phe Asn Gln Thr Trp Ala Thr Phe Ala Met Asn Ile Ala Pro Ala Leu Wal Val Asp Ser Ser Cys Leu Ile Met Asn Leu Gln Ile Lys Thr Tyr Gly Gln Gly Ser Gly Asn Ala Ala Thr Phe Ile Asn Asn His Leu Leu Ser Thr Leu Val Leu Asp Gln Trp Asn Leu Met Arg Gln Pro Arg Pro Asp Ser Glu Glu Phe Lys Ser Ile Glu Asp Lys Leu Gly Ile Asn Phe Lys Ile Glu Arg Ser Ile Asp Asp Ile Arg Gly Lys Leu Arg Gln Leu Val Leu Leu Ala Gln Pro Gly Tyr Leu Ser Gly Gly Val Glu Pro Glu Gln Ser Ser Pro Thr Val Glu Leu Asp Leu Leu Gly Trp Ser Ala Thr Tyr Ser Lys Asp Leu Gly Ile Tyr Val Pro Val Leu Asp Lys Glu Arg

Leu Phe Cys Ser Ala Ala Tyr Pro Lys Gly Val Glu Asn Lys Ser Leu 595 Lys Ser Lys Val Gly Ile Glu Gln Ala Tyr Lys Val Val Arg Tyr Glu Ala Leu Arg Leu Val Gly Gly Trp Asn Tyr Pro Leu Leu Asn Lys Ala 630 635 Cys Lys Asn Asn Ala Gly Ala Ala Arg Arg His Leu Glu Ala Lys Gly 650 Phe Pro Leu Asp Glu Phe Leu Ala Glu Trp Ser Glu Leu Ser Glu Phe 660 Gly Glu Ala Phe Glu Gly Phe Asn Ile Lys Leu Thr Val Thr Ser Glu 675 680 Ser Leu Ala Glu Leu Asn Lys Pro Val Pro Pro Lys Pro Pro Asn Val Asn Arg Pro Val Asn Thr Gly Gly Leu Lys Ala Val Ser Asn Ala Leu 710 715 Tys Thr Gly Arg Tyr Arg Asn Glu Ala Gly Leu Ser Gly Leu Val Leu 725 735 Leu Ala Thr Ala Arg Ser Arg Leu Gln Asp Ala Val Lys Ala Lys Ala Clu Ala Glu Lys Leu His Lys Ser Lys Pro Asp Asp Pro Asp Ala Asp 755 Trp Phe Glu Arg Ser Glu Thr Leu Ser Asp Leu Leu Glu Lys Ala Asp 770 Ile Ala Ser Lys Val Ala His Ser Ala Leu Val Glu Thr Ser Asp Ala 785 795 Leu Glu Ala Val Gln Ser Thr Ser Val Tyr Thr Pro Lys Tyr Pro Glu Val Lys Asn Pro Gln Thr Ala Ser Asn Pro Val Val Gly Leu His Leu 820 825 830 Pro Ala Lys Arg Ala Thr Gly Val Gln Ala Ala Leu Leu Gly Ala Gly 835 Thr Ser Arg Pro Met Gly Met Glu Ala Pro Thr Arg Ser Lys Asn Ala 855 860

Val	Lys	Met	Ala	Lys	Arg	Arg	Gln	Arg	Gln	Lys	Glu	Ser	Arg
865					870	_		_		875			-

(2)	INFO	ORMA!	rion	FOR	SEQ	ID I	10:	3:								
	(i)	(1 (1 (0	QUENCA) LI B) T' C) S' D) TO	ENGTI YPE: TRANI	H: 32 nucl DEDNI	261] leic ESS:	ase acio sino	pai:	rs							
	(ii)	MOI	LECUI	LE T	YPE:	CDN	A									
	(ix)	(2	ATURI A) NZ B) LO	AME/I			531									
	(xi)	SE	QUEN	CE DI	ESCR	IPTI	ON:	SEQ :	ID N): 3	:					
D G GA'.	racg <i>i</i>	ATC (GGTC'	rgaco	cc c	GGGG	GAGT	C AC	CCGG	GGAC	AGG	CCGT	CAA (GGCC	TTGTTC	60
CAG	GATGO	GGA (CTCC	rcct'	rc Ti	ACAA	CGCT	A TC	ATTG					GAT Asp 5		114
ACA Thr	AAC Asn	GAT Asp	CGC Arg 10	AGC Ser	GAT Asp	GAC Asp	AAA Lys	CCT Pro 15	GCA Ala	AGA Arg	TCA Ser	AAC Asn	CCA Pro 20	ACA Thr	GAT Asp	162
TGT Cys	TCC Ser	GTT Val 25	CAT His	ACG Thr	GAG Glu	CCT Pro	TCT Ser 30	GAT Asp	GCC Ala	AAC Asn	AAC Asn	CGG Arg 35	ACC Thr	GGC Gly	GTC Val	210
CAT His	TCC Ser 40	GGA Gly	CGA Arg	CAC His	CCT Pro	GGA Gly 45	GAA Glu	GCA Ala	CAC His	TCT Ser	CAG Gln 50	GTC Val	AGA Arg	GAC Asp	CTC Leu	258
GAC Asp 55	CTA Leu	CAA Gln	TTT Phe	GAC Asp	TGT Cys 60	GGG Gly	GGA Gly	CAC His	AGG Arg	GTC Val 65	AGG Arg	GCT Ala	AAT Asn	TGT Cys	CTT Leu 70	306

TTT CCC TGG ATT CCC TGG CTC AAT TGT GGG TGC TCA CTA CAC ACT GCA

Phe Pro Trp Ile Pro Trp Leu Asn Cys Gly Cys Ser Leu His Thr Ala 75 80 85

354

GGG CAA TG Gly Gln Tr			Arg Se				Asp				402
CCT ACC GG Pro Thr Gl 10	y Gln Leu										450
AGT GAG GI Ser Glu Va 120	C AAG CAC Lys His	ACT TCC Thr Ser 125	TGG TG Trp Tr	G CGT p Arg	TTA Leu	TGC Cys 130	ACT Thr	AAA Lys	CGG Arg	CAC His	498
CAT AAA CG His Lys Ar 135	C CGT GAC g Arg Asp	CTT CCA Leu Pro 140	AGG AA Arg Ly	G CCT s Pro	GAG Glu 145	TGA	ACTGA	CA (GATGT	TAGCT	551
ACAATGGGTT	GATGTCTGC	A ACAGC	CAACA T	CAACG	ACAA	AATI	rggga	AC (GTCCI	AGTAG	611
GGGAAGGGGT	CACCGTCCT	C AGCTT	ACCCA C	ATCAT	ATGA	TCTT	GGGT.	AT (GTGAG	GCTTG	671
GTGACCCCAT	TCCCGCAAT	'A GGGCT	TGACC C	'AAAAA	TGGT	AGC	CACAT	GT (GACAG	CAGTG	731
ACAGGCCCAG	AGTCTACAC	C ATAAC	TGCAG C	CGATG	ATTA	CCAA	ATTCT	CA :	TCAC#	GTACC	791
AACCAGGTGG	GGTAACAAT	C ACACT	GTTCT C	AGCCA	ACAT	TGAT	IGCCA	TC A	ACAAG	CCTCA	851
G CGTTGGGGG	AGAGCTCGT	G TTTCA	AACAA G	CGTCC	ACGG	CCTT	GTAC	TG (GGCGC	CACCA	911
PCTACCTCAT	AGGCTTTG	T GGGAC	AACGG T	AATCA	CCAG	GGCT	rgtgg	CC (GCAA <i>I</i>	CAATG	971
EGCTGACGAC	CGGCACCGA	C AACCT	TATGC C	ATTCA	ATCT	TGT	SATTC	CA A	ACAAZ	CGAGA	1031
TAACCCAGCC	AATCACATO	C ATCAA	ACTGG A	GATAG'	TGAC	CTCC	CAAAA	GT (GGTGG	STCAGG	1091
CAGGGGATCA	GATGTCATO	G TCGGC	AAGAG G	GAGCC'	TAGC	AGT	SACGA	TC (CATG	TGGCA	1151
ACTATCCAGG	GGCCCTCCG	T CCCGT	CACGC T	AGTGG	CCTA	CGA	AGAG	TG (GCAAC	CAGGAT	1211
CCGTCGTTAC	GGTCGCTGG	G GTGAG	CAACT T	CGAGC'	TGAT	CCCA	AATC	CT (GAACI	AGCAA	1271
AGAACCTGGT	TACAGAATA	C GGCCG	ATTTG A	CCCAG	GAGC	CATO	AACT.	AC A	ACAAA	ATTGA	1331
TACTGAGTGA	GAGGGACCG	T CTTGG	CATCA A	GACCG'	TCTG	GCCA	ACAA	GG (GAGTA	CACTG	1391
ACTTTCGTGA	ATACTTCAT	G GAGGT	GGCCG A	CCTCA	ACTC	TCCC	CTGA	AG A	ATTGO	CAGGAG	1451
CATTCGGCTT	CAAAGACAT	A ATCCG	GGCCA T	AAGGA	GGAT	AGCI	GTGC	CG (GTGGI	CTCCA	1511
CATTGTTCCC	ACCTGCCGC	T CCCCT	AGCCC A	TGCAA	FTGG	GGAA	\GGTG'	TA (GACTA	CCTGC	1571
TGGGCGATGA	GGCACAGGC	T GCTTC	AGGAA C	TGCTC	GAGC	CGCG	TCAG	GA A	AAAGO	CAAGAG	1631

CTGCCTCAGG	CCGCATAAGG	CAGCTGACTC	TCGCCGCCGA	CAAGGGGTAC	GAGGTAGTCG	1691
CGAATCTATI	CCAGGTGCCC	CAGAATCCCG	TAGTCGACGG	GATTCTTGCT	TCACCTGGGG	1751
TACTCCGCGG	TGCACACAAC	CTCGACTGCG	TGTTAAGAGA	GGGTGCCACG	CTATTCCCTG	1811
TGGTTATTAC	GACAGTGGAA	GACGCCATGA	CACCCAAAGC	ATTGAACAGC	AAAATGTTTG	1871
CTGTCATTGA	AGGCGTGCGA	GAAGACCTCC	AACCTCCATC	TCAAAGAGGA	TCCTTCATAC	1931
GAACTCTCTC	TGGACACAGA	GTCTATGGAT	ATGCTCCAGA	TGGGGTACTT	CCACTGGAGA	1991
CTGGGAGAGA	CTACACCGTT	GTCCCAATAG	ATGATGTCTG	GGACGACAGC	ATTATGCTGT	2051
CCAAAGATCC	CATACCTCCT	ATTGTGGGAA	ACAGTGGAAA	TCTAGCCATA	GCTTACATGG	2111
ATGTGTTTCG	ACCCAAAGTC	CCAATCCATG	TGGCTATGAC	GGGAGCCCTC	AATGCTTGTG	2171
GCGAGATTGA	GAAAGTAAGC	TTTAGAAGCA	CCAAGCTCGC	CACTGCACAC	CGACTTGGCC	2231
TAGGTTGGC	TGGTCCCGGA	GCATTCGATG	TAAACACCGG	GCCCAACTGG	GCAACGTTCA	2291
TCAAACGTTT	CCCTCACAAT	CCACGCGACT	GGGACAGGCT	CCCCTACCTC	AACCTACCAT	2351
ACCTTCCACC	CAATGCAGGA	CGCCAGTACC	ACCTTGCCAT	GGCTGCATCA	GAGTTCAAAG	2411
AGACCCCCGA	ACTCGAGAGT	GCCGTCAGAG	CAATGGAAGC	AGCAGCCAAC	GTGGACCCAC	2471
PATTCCAATC	TGCACTCAGT	GTGTTCATGT	GGCTGGAAGA	GAATGGGATT	GTGACTGACA	2531
TGGCCAACTT	CGCACTCAGC	GACCCGAACG	CCCATCGGAT	GCGAAATTTT	CTTGCAAACG	2591
CACCACAAGC	AGGCAGCAAG	TCGCAAAGGG	CCAAGTACGG	GACAGCAGGC	TACGGAGTGG	2651
AGGCTCGGGG	CCCCACACCA	GAGGAAGCAC	AGAGGGAAAA	AGACACACGG	ATCTCAAAGA	2711
AGATGGAGAC	CATGGGCATC	TACTTTGCAA	CACCAGAATG	GGTAGCACTC	AATGGGCACC	2771
GAGGGCCAAG	CCCCGGCCAG	CTAAAGTACT	GGCAGAACAC	ACGAGAAATA	CCGGACCCAA	2831
ACGAGGACTA	TCTAGACTAC	GTGCATGCAG	AGAAGAGCCG	GTTGGCATCA	GAAGAACAAA	2891
TCCTAAGGGC	AGCTACGTCG	ATCTACGGGG	CTCCAGGACA	GGCAGAGCCA	CCCCAAGCTT	2951
TCATAGACGA	AGTTGCCAAA	GTCTATGAAA	TCAACCATGG	ACGTGGCCCA	AACCAAGAAC	3011
AGATGAAAGA	TCTGCTCTTG	ACTGCGATGG	AGATGAAGCA	TCGCAATCCC	AGGCGGGCTC	3071
TACCAAAGCC	CAAGCCAAAA	CCCAATGCTC	CAACACAGAG	ACCCCCTGGT	CGGCTGGGCC	3131
GCTGGATCAG	GACCGTCTCT	GATGAGGACC	TTGAGTGAGG	CTCCTGGGAG	TCTCCCGACA	3191

CCACCCGCGC AGGTGTGGAC ACCAATTCGG CCTTACAACA TCCCAAATTG GATCCGTTCG
CGGGTCCCCT

3251

3261

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 145 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Val Ser Arg Asp Gln Thr Asn Asp Arg Ser Asp Asp Lys Pro Ala 1 5 10 15

Arg Ser Asn Pro Thr Asp Cys Ser Val His Thr Glu Pro Ser Asp Ala
20 25 30

Asn Asn Arg Thr Gly Val His Ser Gly Arg His Pro Gly Glu Ala His

35
40
45

Ser Gln Val Arg Asp Leu Asp Leu Gln Phe Asp Cys Gly Gly His Arg
50 55 60

Wal Arg Ala Asn Cys Leu Phe Pro Trp Ile Pro Trp Leu Asn Cys Gly
75 70 80

Cys Ser Leu His Thr Ala Gly Gln Trp Glu Leu Gln Val Arg Ser Asp

Ala Pro Asp Cys Pro Glu Pro Thr Gly Gln Leu Gln Leu Gln Ala 100 105 110

Ser Glu Ser Glu Ser His Ser Glu Val Lys His Thr Ser Trp Trp Arg 115 120 125

Leu Cys Thr Lys Arg His His Lys Arg Arg Asp Leu Pro Arg Lys Pro
130 135 140

Glu

145

(2) INFORMATION FOR SEQ ID NO: 5:

- (A) LENGTH: 3261 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 131..3166

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGATACO	ATC	GGTC'	TGAC(cc c	GGGG	GAGT	C AC	CCGG	GGAC	AGG	CCGT	CAA (GCC	TTGTTC	60
©AGGATO	GGA	CTCC'	rcct:	rc T	ACAA	CGCT	A TC	ATTG	ATGG	TTA	GTAG	AGA (rcag?	ACAAAC	120
EATCGCZ F C)		ATG A Met 1													169
TTC ATA Phe Ile 15	Arg														217
Asp Asp	ACC Thr	CTG Leu	GAG Glu	AAG Lys 35	CAC His	ACT Thr	CTC Leu	AGG Arg	TCA Ser 40	GAG Glu	ACC Thr	TCG Ser	ACC Thr	TAC Tyr 45	265
AAT TTO Asn Lev															313
GGA TTO	CCT Pro	GGC Gly 65	TCA Ser	ATT Ile	GTG Val	GGT Gly	GCT Ala 70	CAC His	TAC Tyr	ACA Thr	CTG Leu	CAG Gln 75	GGC Gly	AAT Asn	361
GGG AAC Gly Asr	TAC Tyr 80	AAG Lys	TTC Phe	GAT Asp	CAG Gln	ATG Met 85	CTC Leu	CTG Leu	ACT Thr	GCC Ala	CAG Gln 90	AAC Asn	CTA Leu	CCG Pro	409
GCC AGT Ala Ser 95	Tyr	AAC Asn	TAC Tyr	TGC Cys	AGG Arg 100	CTA Leu	GTG Val	AGT Ser	CGG Arg	AGT Ser 105	CTC Leu	ACA Thr	GTG Val	AGG Arg	457

TCA	AGC Ser	ACA	CTT	CCT	GGT	GGC	COO	m v m	CON	Om 3	770	ccc	7.00	3 00 3	330	
110		Thr	Leu	Pro	Gly 115	Gly	Val	Tyr	Ala	Leu 120	Asn	Gly	Thr	Ile	Asn 125	505
GCC Ala	GTG Val	ACC Thr	TTC Phe	CAA Gln 130	GGA Gly	AGC Ser	CTG Leu	AGT Ser	GAA Glu 135	CTG Leu	ACA Thr	GAT Asp	GTT Val	AGC Ser 140	TAC Tyr	553
AAT Asn	GGG Gly	TTG Leu	ATG Met 145	TCT Ser	GCA Ala	ACA Thr	GCC Ala	AAC Asn 150	ATC Ile	AAC Asn	GAC Asp	AAA Lys	ATT Ile 155	GGG Gly	AAC Asn	601
GTC Val	CTA Leu	GTA Val 160	GGG Gly	GAA Glu	GGG Gly	GTC Val	ACC Thr 165	GTC Val	CTC Leu	AGC Ser	TTA Leu	CCC Pro 170	ACA Thr	TCA Ser	TAT Tyr	649
GAT Asp	CTT Leu 175	GGG Gly	TAT Tyr	GTG Val	AGG Arg	CTT Leu 180	GGT Gly	GAC Asp	CCC Pro	ATT Ile	CCC Pro 185	GCA Ala	ATA Ile	GGG Gly	CTT Leu	697
GAC Asp 190	CCA Pro	AAA Lys	ATG Met	GTA Val	GCC Ala 195	ACA Thr	TGT Cys	GAC Asp	AGC Ser	AGT Ser 200	GAC Asp	AGG Arg	CCC Pro	AGA Arg	GTC Val 205	745
ĒĀC Tyr	ACC Thr	ATA Ile	ACT Thr	GCA Ala 210	GCC Ala	GAT Asp	GAT Asp	TAC Tyr	CAA Gln 215	TTC Phe	TCA Ser	TCA Ser	CAG Gln	TAC Tyr 220	CAA Gln	793
eca Pro	GGT Gly	GGG Gly	GTA Val 225	ACA Thr	ATC Ile	ACA Thr	CTG Leu	TTC Phe 230	TCA Ser	GCC Ala	AAC Asn	ATT Ile	GAT Asp 235	GCC Ala	ATC Ile	841
ACA Thr	AGC Ser	CTC Leu 240	AGC Ser	GTT Val	GGG Gly	GGA Gly	GAG Glu 245	CTC Leu	GTG Val	TTT Phe	CAA Gln	ACA Thr 250	AGC Ser	GTC Val	CAC His	889
GGC Gly	CTT Leu 255	GTA Val	CTG Leu	GGC Gly	GCC Ala	ACC Thr 260	ATC Ile	TAC Tyr	CTC Leu	ATA Ile	GGC Gly 265	TTT Phe	GAT Asp	GGG Gly	ACA Thr	937
ACG Thr 270	GTA Val	ATC Ile	ACC Thr	AGG Arg	GCT Ala 275	GTG Val	GCC Ala	GCA Ala	AAC Asn	AAT Asn 280	GGG Gly	CTG Leu	ACG Thr	ACC Thr	GGC Gly 285	985
ACC Thr	GAC Asp	AAC Asn	CTT Leu	ATG Met 290	CCA Pro	TTC Phe	AAT Asn	CTT Leu	GTG Val 295	ATT Ile	CCA Pro	ACA Thr	AAC Asn	GAG Glu 300	ATA Ile	1033
ACC Thr	CAG Gln	CCA Pro	ATC Ile 305	ACA Thr	TCC Ser	ATC Ile	AAA Lys	CTG Leu 310	GAG Glu	ATA Ile	GTG Val	ACC Thr	TCC Ser 315	AAA Lys	AGT Ser	1081

					GAT Asp											1129
					GGT Gly											1177
					GAA Glu 355											1225
					TTC Phe											1273
					TAC Tyr											1321
					AGT Ser											1369
IGG					TAC Tyr											1417
					CCC Pro 435											1465
					ATA Ile											1513
TTG Leu	TTC Phe	CCA Pro	CCT Pro 465	GCC Ala	GCT Ala	CCC Pro	CTA Leu	GCC Ala 470	CAT His	GCA Ala	ATT Ile	GGG Gly	GAA Glu 475	GGT Gly	GTA Val	1561
					GAT Asp											1609
GCC Ala	GCG Ala 495	TCA Ser	GGA Gly	AAA Lys	GCA Ala	AGA Arg 500	GCT Ala	GCC Ala	TCA Ser	GGC Gly	CGC Arg 505	ATA Ile	AGG Arg	CAG Gln	CTG Leu	1657
ACT Thr 510	CTC Leu	GCC Ala	GCC Ala	GAC Asp	AAG Lys 515	GGG Gly	TAC Tyr	GAG Glu	GTA Val	GTC Val 520	GCG Ala	AAT Asn	CTA Leu	TTC Phe	CAG Gln 525	1705

							CCT Pro		1753
							GGT Gly 555		1801
							ACA Thr		1849
							CGA Arg		1897
							CTC Leu		1945
CAC							CTG Leu		1993
ĢĒG							GAC Asp 635		2041
							AAC Asn		2089
							GTC Val		2137
							ATT Ile		2185
							CTT Leu		2233
							CCC Pro 715		2281
							TGG Trp		2329

				AAC Asn												2377
				ATG Met												2425
				AGA Arg 770												2473
				CTC Leu												2521
				GCC Ala												2569
				CTT Leu												2617
AGG				GGG Gly												2665
				GCA Ala 850												2713
ATG Met	GAG Glu	ACC Thr	ATG Met 865	GGC Gly	ATC Ile	TAC Tyr	TTT Phe	GCA Ala 870	ACA Thr	CCA Pro	GAA Glu	TGG Trp	GTA Val 875	GCA Ala	CTC Leu	2761
_				GGG Gly												2809
ACA Thr	CGA Arg 895	GAA Glu	ATA Ile	ccg Pro	GAC Asp	CCA Pro 900	AAC Asn	GAG Glu	GAC Asp	TAT Tyr	CTA Leu 905	GAC Asp	TAC Tyr	GTG Val	CAT His	2857
GCA Ala 910	GAG Glu	AAG Lys	AGC Ser	CGG Arg	TTG Leu 915	GCA Ala	TCA Ser	GAA Glu	GAA Glu	CAA Gln 920	ATC Ile	CTA Leu	AGG Arg	GCA Ala	GCT Ala 925	2905
ACG Thr	TCG Ser	ATC Ile	TAC Tyr	GGG Gly 930	GCT Ala	CCA Pro	GGA Gly	CAG Gln	GCA Ala 935	GAG Glu	CCA Pro	CCC Pro	CAA Gln	GCT Ala 940	TTC Phe	2953

.

			GTT Val 945													3001
			CAG Gln													3049
			CCC Pro													3097
			CAG Gln								Arg					3145
			GAG Glu		Leu		TGAC	GCTO	CCT (GGAC	GTCT(cc co	GACA	CCAC		3196
-75	GCAG	GTG :	rgga	CACC	AA T	rcggo	CTTA	A CA	ACATO	CCCA	AAT	rggar	rcc (GTTC	GCGGGT	3256
CCC (2)		OR M A'	rion	FOR	SEQ	ID 1	10: (5:								3261
	l	(1 (1	SEQUI A) LI 3) TY	ENGTI	4: 10 amir)12 a	amino cid									
			LECUI QUEN					SEQ I	ED NO	D: 6:	:					
Met 1	Thr	Asn	Leu			Gln								Ile 15	Arg	
Ser	Leu	Leu	Met 20	Pro	Thr	Thr	Gly	Pro 25	Ala	Ser	Ile	Pro	Asp 30	Asp	Thr	
Leu	Glu	Lys 35	His	Thr	Leu	Arg	Ser 40	Glu	Thr	Ser	Thr	Tyr 45	Asn	Leu	Thr	
Val	Gly 50	Asp	Thr	Gly	Ser	Gly 55	Leu	Ile	Val	Phe	Phe 60	Pro	Gly	Phe	Pro	
Gly 65	Ser	Ile	Val	Gly	Ala 70	His	Tyr	Thr	Leu	Gln 75	Gly	Asn	Gly	Asn	Tyr 80	

Lys Phe Asp Gln Met Leu Leu Thr Ala Gln Asn Leu Pro Ala Ser Tyr Asn Tyr Cys Arq Leu Val Ser Arg Ser Leu Thr Val Arg Ser Ser Thr Leu Pro Gly Gly Val Tyr Ala Leu Asn Gly Thr Ile Asn Ala Val Thr Phe Gln Gly Ser Leu Ser Glu Leu Thr Asp Val Ser Tyr Asn Gly Leu Met Ser Ala Thr Ala Asn Ile Asn Asp Lys Ile Gly Asn Val Leu Val Gly Glu Gly Val Thr Val Leu Ser Leu Pro Thr Ser Tyr Asp Leu Gly Tyr Val Arg Leu Gly Asp Pro Ile Pro Ala Ile Gly Leu Asp Pro Lys Met Val Ala Thr Cys Asp Ser Ser Asp Arg Pro Arg Val Tyr Thr Ile Thr Ala Ala Asp Asp Tyr Gln Phe Ser Ser Gln Tyr Gln Pro Gly Gly ∀al Thr Ile Thr Leu Phe Ser Ala Asn Ile Asp Ala Ile Thr Ser Leu ser Val Gly Gly Glu Leu Val Phe Gln Thr Ser Val His Gly Leu Val Leu Gly Ala Thr Ile Tyr Leu Ile Gly Phe Asp Gly Thr Thr Val Ile Thr Arg Ala Val Ala Ala Asn Asn Gly Leu Thr Thr Gly Thr Asp Asn Leu Met Pro Phe Asn Leu Val Ile Pro Thr Asn Glu Ile Thr Gln Pro Ile Thr Ser Ile Lys Leu Glu Ile Val Thr Ser Lys Ser Gly Gly Gln Ala Gly Asp Gln Met Ser Trp Ser Ala Arg Gly Ser Leu Ala Val Thr Ile His Gly Gly Asn Tyr Pro Gly Ala Leu Arg Pro Val Thr Leu Val

Ala Tyr Glu Arg Val Ala Thr Gly Ser Val Val Thr Val Ala Gly Val Ser Asn Phe Glu Leu Ile Pro Asn Pro Glu Leu Ala Lys Asn Leu Val Thr Glu Tyr Gly Arg Phe Asp Pro Gly Ala Met Asn Tyr Thr Lys Leu Ile Leu Ser Glu Arg Asp Arg Leu Gly Ile Lys Thr Val Trp Pro Thr Arg Glu Tyr Thr Asp Phe Arg Glu Tyr Phe Met Glu Val Ala Asp Leu Asn Ser Pro Leu Lys Ile Ala Gly Ala Phe Gly Phe Lys Asp Ile Ile Arg Ala Ile Arg Arg Ile Ala Val Pro Val Val Ser Thr Leu Phe Pro Pro Ala Ala Pro Leu Ala His Ala Ile Gly Glu Gly Val Asp Tyr Leu Leu Gly Asp Glu Ala Gln Ala Ala Ser Gly Thr Ala Arg Ala Ala Ser Gly Lys Ala Arg Ala Ala Ser Gly Arg Ile Arg Gln Leu Thr Leu Ala Ala Asp Lys Gly Tyr Glu Val Val Ala Asn Leu Phe Gln Val Pro Gln Asn Pro Val Val Asp Gly Ile Leu Ala Ser Pro Gly Val Leu Arg Gly Ala His Asn Leu Asp Cys Val Leu Arg Glu Gly Ala Thr Leu Phe Pro Val Val Ile Thr Thr Val Glu Asp Ala Met Thr Pro Lys Ala Leu Asn Ser Lys Met Phe Ala Val Ile Glu Gly Val Arg Glu Asp Leu Gln Pro Pro Ser Gln Arg Gly Ser Phe Ile Arg Thr Leu Ser Gly His Arg Val Tyr Gly Tyr Ala Pro Asp Gly Val Leu Pro Leu Glu Thr Gly Arg Asp

Tyr Thr Val Val Pro Ile Asp Asp Val Trp Asp Asp Ser Ile Met Leu Ser Lys Asp Pro Ile Pro Pro Ile Val Gly Asn Ser Gly Asn Leu Ala Ile Ala Tyr Met Asp Val Phe Arg Pro Lys Val Pro Ile His Val Ala Met Thr Gly Ala Leu Asn Ala Cys Gly Glu Ile Glu Lys Val Ser Phe Arg Ser Thr Lys Leu Ala Thr Ala His Arg Leu Gly Leu Arg Leu Ala Gly Pro Gly Ala Phe Asp Val Asn Thr Gly Pro Asn Trp Ala Thr Phe Lle Lys Arg Phe Pro His Asn Pro Arg Asp Trp Asp Arg Leu Pro Tyr heu Asn Leu Pro Tyr Leu Pro Pro Asn Ala Gly Arg Gln Tyr His Leu 🖺 a Met Ala Ala Ser Glu Phe Lys Glu Thr Pro Glu Leu Glu Ser Ala Yal Arg Ala Met Glu Ala Ala Ala Asn Val Asp Pro Leu Phe Gln Ser Ala Leu Ser Val Phe Met Trp Leu Glu Glu Asn Gly Ile Val Thr Asp Met Ala Asn Phe Ala Leu Ser Asp Pro Asn Ala His Arg Met Arg Asn Phe Leu Ala Asn Ala Pro Gln Ala Gly Ser Lys Ser Gln Arg Ala Lys Tyr Gly Thr Ala Gly Tyr Gly Val Glu Ala Arg Gly Pro Thr Pro Glu Glu Ala Gln Arg Glu Lys Asp Thr Arg Ile Ser Lys Lys Met Glu Thr Met Gly Ile Tyr Phe Ala Thr Pro Glu Trp Val Ala Leu Asn Gly His Arg Gly Pro Ser Pro Gly Gln Leu Lys Tyr Trp Gln Asn Thr Arg Glu

Ile	Pro	Asp	Pro 900	Asn	Glu	Asp	Tyr	Leu 905	Asp	Tyr	Val	His	Ala 910	Glu	Lys	
Ser	Arg	Leu 915	Ala	Ser	Glu	Glu	Gln 920	Ile	Leu	Arg	Ala	Ala 925	Thr	Ser	Ile	
Tyr	Gly 930	Ala	Pro	Gly	Gln	Ala 935	Glu	Pro	Pro	Gln	Ala 940	Phe	Ile	Asp	Glu	
Val 945	Ala	Lys	Val	Tyr	Glu 950	Ile	Asn	His	Gly	Arg 955	Gly	Pro	Asn	Gln	Glu 960	
Gln	Met	Lys	Asp	Leu 965	Leu	Leu	Thr	Ala	Met 970	Glu	Met	Lys	His	Arg 975	Asn	
Pro	Arg	Arg	Ala 980	Leu	Pro	Lys	Pro	Lys 985	Pro	Lys	Pro	Asn	Ala 990	Pro	Thr	
ter.	Arg	Pro 995		Gly	Arg	Leu	Gly 100	Arg 0	Trp	Ile	Arg	Thr 100	Val 5	Ser	Asp	
2222	elu Asp Leu Glu 1010															
(2)	INF	ORMA	TION	FOR	SEQ	ID	NO:	7:								
	(i	· (A) L B) T C) S	ENGT YPE: TRAN	HARA H: 3 nuc DEDN	261 leic ESS:	base aci sin	pai d	rs							
ਜੈਨ ਦੇ ਨੂੰ ਦੇ ਹੈ ਜੈਨਾਵਾਂ ਜੈਨਾਵਾਂ	(ii) MO	LECU	LE I	YPE:	cDN	Ά									
	(ix	· (ATUR A) N B) I	IAME/	KEY:	CDS	531									
	(xi) SE	EQUE	ICE I	ESCF	RIPTI	ON:	SEQ	ID N	10: 7	7:					
GGI	ATACO	SATC	GGT	CTGAC	ecc c	GGGG	GAGI	C AC	CCGG	GGAC	C AGO	CCG	CAA	GGCC	CTTGTTC	60
CAC	GATO	GGA	CTC	CTCC	TC I	ACA	ACGCI	T AT	CATTO	GA/	A GTT	r AG	TG!	GAT	CTG	114

Glu Val Ser * Asp Leu
1 5

ACA Thr	AAC (Asn .	GAT (Asp i	CGC A Arg :	AGC (Ser)	GAT (GAC Asp	AAA Lys	CCT Pro 15	GCA Ala	AGA Arg	TCA Ser	AAC Asn	CCA Pro 20	ACA Thr	GAT Asp	162
(2)	INFO	R MAT	ION :	FOR	SEQ	ID N	0: 8	:								
	(i)	(B (C	UENC) LE) TY) ST) TO	NGTH PE: RAND	: 28 nucl EDNE	27 b eic SS:	ase acid sing	pair l	:s							
	(ii)	MOL	ECUL	E TY	PE:	cDNA	•									
	(ix)		TURE) NA) LO	ME/K			2745	5								
	(xi)	SEQ	UENC	E DE	SCRI	PTIC	on: S	SEQ I	ED NO) : 8:	•					
G GA'	racg <i>a</i>	TG G	GTCT	GACC	C TO	TGG	AGTO	C AC	GAAT	TAAC	GTG	GCTA	CTA	GGGG	CGATAC	60
H 15.5	CCGCI	rgg c	CTGCC	CACGI	T AC	TGG	CTCC	r ct	rctt(GATG	ATT	CTGC	CAC	C AT Me	G AGT t Ser 1	117
€AC ≜sp	ATT Ile	TTC Phe 5	AAC Asn	AGT Ser	CCA Pro	CAG Gln	GCG Ala 10	CGA Arg	AGC Ser	ACG Thr	ATC	TCA Ser 15	Ala	GCG Ala	TTC Phe	165
GGC Gly	ATA Ile 20	AAG Lys	CCT Pro	ACT Thr	GCT Ala	GGA Gly 25	CAA Gln	GAC Asp	GTG Val	GAA Glu	GAA Glu 30	Leu	TTG Leu	ATC	CCT Pro	213
AAA Lys 35	Val	TGG Trp	GTG Val	CCA Pro	CCT Pro 40	GAG Glu	GAT Asp	CCG Pro	CTT Leu	GCC Ala 45	Ser	C CCT	AGT Ser	CGA Arg	CTG Leu 50	261
GCA Ala	AAG Lys	TTC Phe	CTC Leu	AGA Arg 55	GAG Glu	AAC Asn	GGC Gly	TAC Tyr	AAA Lys 60	Val	TTC Lev	CAG Gln	CCG Pro	G CGG Arg	TCT Ser	309
CTG Leu	CCC	GAG Glu	AAT Asn 70	GAG Glu	GAG Glu	TAT Tyr	GAG Glu	ACC Thr	Asp	CAA Gln	ATA	A CTO	C CCA Pro 80) Asp	TTA Leu	357

GCA Ala	TGG Trp	ATG Met 85	CGA Arg	CAG Gln	ATA Ile	GAA Glu	GGG Gly 90	GCT Ala	GTT Val	TTA Leu	AAA Lys	CCC Pro 95	ACT Thr	CTA Leu	TCT Ser	405
CTC Leu	CCT Pro 100	ATT Ile	GGA Gly	GAT Asp	CAG Gln	GAG Glu 105	TAC Tyr	TTC Phe	CCA Pro	AAG Lys	TAC Tyr 110	TAC Tyr	CCA Pro	ACA Thr	CAT His	453
CGC Arg 115	CCT Pro	AGC Ser	AAG Lys	GAG Glu	AAG Lys 120	CCC Pro	AAT Asn	GCG Ala	TAC Tyr	CCG Pro 125	CCA Pro	GAC Asp	ATC Ile	GCA Ala	CTA Leu 130	501
CTC Leu	AAG Lys	CAG Gln	ATG Met	ATT Ile 135	TAC Tyr	CTG Leu	TTT Phe	CTC Leu	CAG Gln 140	GTT Val	CCA Pro	GAG Glu	GCC Ala	AAC Asn 145	GAG Glu	549
GGC Gly	CTA Leu	AAG Lys	GAT Asp 150	GAA Glu	GTA Val	ACC Thr	CTC Leu	TTG Leu 155	Thr	CAA Gln	AAC Asn	ATA Ile	AGG Arg 160	GAC Asp	AAG Lys	597
1	TAT Tyr	GGA Gly 165	Ser	GGG Gly	ACC Thr	TAC Tyr	ATG Met 170	GIY	CAA Gln	GCA Ala	ACT Thr	CGA Arg 175	CTT Leu	GTG Val	GCC Ala	645
Nmc	AAG Lys 180	Glu	GTC Val	GCC Ala	ACT Thr	GGA Gly 185	AGA Arg	AAC Asn	CCA Pro	AAC Asn	AAG Lys 190	Asp	CCT Pro	CTA Leu	AAG Lys	693
L eu 195	Gly	TAC Tyr	ACT Thr	TTT Phe	GAG Glu 200	Ser	ATC Ile	GCG Ala	CAG Gln	CTA Leu 205	Leu	GAC Asp	ATC Ile	ACA Thr	CTA Leu 210	741
©CG Pro	GTA Val	GGC Gly	C CCA	CCC Pro 215	Gly	GAG Glu	GAT Asp	GAC Asp	AAC Lys 220	Pro	TGG Trp	GTG Val	CCA Pro	CTC Lev 225	ACA Thr	789
AGA Arg	A GTO	G CCC	G TCA Ser 230	Arg	G ATO	TTC Lev	GTO Val	G CTO L Lev 235	ומדי ג	G GGA	A GA(y As)	C GTA o Val	A GAT L Asp 240	, GT	GAC Asp	837
TTT Phe	r GA(e Gl	G GT' 1 Va: 24	l Glu	A GA! u As)	т ТАС р Туз	C CTI	CCC Pro 25	о Гу	A ATO	C AAG e Asi	C CTO	C AAG u Lys 259	5 Sei	A TC	A AGT	885
GGA Gly	A CT y Lev 26	u Pr	А ТА' о Ту:	r GTA	A GG' l Gly	r CGG y Arg 26!	g Th	C AA r Ly	A GG s Gl	A GA	G AC u Th	L III	r GGG e Gl	C GAO y Gl	G ATG u Met	933
AT 110 27	e Al	T AT. a Il	A TC. e Se	A AA r As	C CAO n Gl: 28	n Ph	r ct e Le	C AG u Ar	A GA g Gl	G CT u Le 28	u se	A AC	A CTO	G TT u Le	G AAG u Lys 290	981

							CTC Leu		1029
							CCA Pro 320		1077
							AAC Asn		1125
							ACC Thr		1173
							GGG Gly		1221
3 5							AGG Arg		1269
GAG Glu									1317
AAC JAsn									1365
AAG Lys									1413
							ATG Met		1461
							CTA Leu		1509
							TAT Tyr 480		1557
		Asn					TTG Leu		1605

					TGG Trp									1653
					ATT Ile 520									1701
					GAT Asp									1749
					TAC Tyr									1797
					CTT Leu									1845
AAA Lys														1893
TGT Cys 595														1941
ZAAA Lys														1989
AGG Arg														2037
	Asn	Ala	Gly	Ala	GCT Ala	Arg	Arg	His	Leu		Lys			2085
					GCC Ala									2133
					AAT Asn 680									2181
					CCA Pro									2229

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			ACT Thr 710													2277
			AGG Arg													2325
			AGC Ser													2373
			CAC His													2421
			GAA Glu													2469
AGC Ser	AAG Lys	GTC Val	GCC Ala 790	CAC His	TCA Ser	GCA Ala	CTC Leu	GTG Val 795	GAA Glu	ACA Thr	AGC Ser	GAC Asp	GCC Ala 800	CTT Leu	GAA Glu	2517
ĢСА	GTT Val	CAG Gln 805	TCG Ser	ACT Thr	TCC Ser	GTG Val	TAC Tyr 810	ACC Thr	CCC Pro	AAG Lys	TAC Tyr	CCA Pro 815	GAA Glu	GTC Val	AAG Lys	2565
			ACC Thr													2613
AAG Lys 835	AGA Arg	GCC Ala	ACC Thr	GGT Gly	GTC Val 840	CAG Gln	GCC Ala	GCT Ala	CTT Leu	CTC Leu 845	GGA Gly	GCA Ala	GGA Gly	ACG Thr	AGC Ser 850	2661
AGA Arg	CCA Pro	ATG Met	GGG Gly	ATG Met 855	GAG Glu	GCC Ala	CCA Pro	ACA Thr	CGG Arg 860	TCC Ser	AAG Lys	AAC Asn	GCC Ala	GTG Val 865	AAA Lys	2709
ATG Met	GCC Ala	AAA Lys	CGG Arg 870	CGG Arg	CAA Gln	CGC Arg	CAA Gln	AAG Lys 875	GAG Glu	AGC Ser	cgc Arg	TAA	CAGC	CAT		2755
GAT	GGGA	ACC A	ACTC	AAGA	AG AC	GAC	CTA	A TCC	CCAG	ACCC	CGT	ATCC	CCG (CCT	CGCCT	2815
GCG	GGGG	ccc o	CC													2827

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Recombinant birnavirus vaccine

The present invention is concerned with a birnavirus mutant, a vaccine comprising this mutant, a method for determining birnavirus infection in an animal, as well as with a test kit for carrying out this method.

Infectious bursal disease virus (IBDV) and Infectious pancreatic necrosis virus (IPNV) are members of the Birnaviridae family. Viruses in this family have a very similar genomic organisation and a similar replication cycle. The genomes of these viruses consist of 2 segments (A and B) of double-stranded (ds) RNA. The larger segment A encodes a polyprotein which is cleaved by autoproteolysis to form mature viral proteins VP2, VP3 and VP4 (Hudson, P.J. et al, Nucleic Acids Res., 14, 5001-50012, 1986; Dobos P., Annual review of fish diseases 5, 25-54, 1995). VP2 and VP3 are the major structural proteins of the virion. VP2 is the major host-protective immunogen of birnaviruses, and contains the antigenic regions responsible for the induction of neutralising antibodies. The VP4 protein appears to be a virus-coded protease that is involved in the processing of a precursor polyprotein of the VP2, VP3 and VP4 proteins. The larger segment A possesses also a second open reading frame (ORF), preceding and partially overlapping the polyprotein gene. This second open reading frame encodes a protein VP5 of unknown function that is present in IBDV infected cells (Mundt, E. et al., J. Gen. Virol., 76, 437-443, 1995).

The smaller segment B encodes VP1, a 90 kDa multifunctional protein with polymerase and capping enzyme activities (Spies, U. et al., Virus Res., 8, 127-140, 1987 and Spies, U. et al., J. Gen. Virol., 71, 977-981, 1990; Duncan R. et al., Virology 181, 541-552, 1991).

For IBDV, two serotypes exist, serotype 1 and 2. The 2 serotypes may be differentiated by virus neutralisation (VN) tests. Furthermore, subtypes of serotype 1 have been isolated. These so-called "variant" viruses of serotype 1 can be identified by cross-neutralisation tests (Diseases of Poultry, 9th edition, 1991, Wolfe Publishing Ltd, ISBN 0 7234 1706 7, Chapter 28, P.D. Lukert and Y.M. Saif, 648-663), a panel of monoclonal antibodies (Snyder, D.B. et al., Arch. Virol., 127, 89-101. 1992.) or RT-PCR (Jackwood, D.J., Proceedings of the International symposium on infectious bursal disease and chicken infectious anaemia, Rauischholzhausen, Germany, 155-161, 1994). Some of these subtypes of serotype 1 of IBDV have been described in literature for example: Classical, Variant-E, GLS, RS593 and DS326 strains (Van Loon, et

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al. Proceedings of the International symposium on infectious bursal disease and chicken infectious anaemia, Rauischholzhausen, Germany, 179-187, 1994).

Infectious Bursal disease (IBD), also called Gumboro disease, is an acute, highly-contagious viral infection in chickens that has lymphoid tissue as its primary target with a selective tropism for cells of the bursa of Fabricius. The morbidity rate in susceptible flocks is high, with rapid weight loss and moderate mortality rates. Chicks that recover from the disease may have immune deficiencies because of the destruction of the bursa of Fabricius which is essential to the defence mechanism of the chicken. The IBD-virus causes severe immunosuppression in chickens younger than 3 weeks of age and induces bursal lesions in chicks up to 3 months old.

For many years the disease could be prevented by inducing high levels of antibodies in breeder flocks by the application of an inactivated vaccine, to chickens that had been primed with attenuated live IBDV vaccine. This has kept economic losses caused by IBD to a minimum. Maternal antibodies in chickens derived from vaccinated breeders prevents early infection with IBDV and diminishes problems associated with immunosuppression. In addition, attenuated live vaccines have also been used successfully in commercial chicken flocks after maternal antibodies had declined.

Recently, very virulent strains of IBDV have caused outbreaks of disease with high mortality in Europe. The current vaccination programs failed to protect chicks sufficiently. Vaccination failures were mainly due to the inability of live vaccines to infect the birds before challenge with virulent field virus.

Eradication of the disease by other preventative measures than vaccination has not been feasible, because the virus is widely spread and because with currently administered live attenuated or inactivated IBDV vaccines it is not possible to determine whether a specific animal is infected with an IBDV field virus or whether the animal was vaccinated with an IBDV vaccine. In order to be able to start an eradication control programme for IBDV it is highly desirable that the possibility exists to discriminate between animals vaccinated with an IBDV vaccine and those infected with a field virus so as to be able to take appropriate measures, i.e. remove infected flocks, to reduce spreading of the virulent field virus. The introduction of, for example, a serologically identifiable marker can be achieved by introducing

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a mutation in genes encoding non-essential (glyco)proteins of the IBDV which still give rise to the production of antibodies in an infected host animal. A marker vaccine for Aujeszky's disease and companion diagnostic tests have proven their practical value in the control of this disease. Whereas such control programs for other viral infectious diseases in animals are under development, until the present invention a vaccine based on an IBDV vaccine strain which would fit in IBDV control programs has not been described yet. The main reason for this is that the prerequisites for the development for such an IBDV marker vaccine were not met. No permissive position or region in the genomic IBDV sequence, i.e. a position or region which can be used for the incorporation of the mutation without disrupting essential functions of IBDV, such as those necessary for infection and replication, have been identified yet. Moreover, such a non-essential region in the IBDV genome should encode a (glyco)protein which elicits a major serological response in an animal infected with wild-type IBDV, and such a region was not identified before.

The present inventors have unexpectedly found a non-essential gene within segment A of a birnavirus genome which can be mutated such that the resulting birnavirus mutant does not produce the native expression product of that gene. Moreover, it has been found that this birnavirus mutant can be used as a marker vaccine virus which allows to make a serological distinction between animals infected with wild-type birnavirus and animals immunised with a vaccine based on this birnavirus mutant.

The present invention provides a birnavirus mutant which is not able to produce a native VP5 protein as a result of a mutation in the VP5 gene of the birnavirus genome.

Preferably, the birnavirus mutant is an IBDV mutant or an IPNV mutant, the IBDV mutant being most preferred, in particular an IBDV mutant derived from a serotype 1 IBD virus is provided by the present invention.

The inventors have found that an IBDV mutant which is not able to produce the native VP5 protein is still able to infect cells and to replicate in these cells <u>in vitro</u>. It is demonstrated that the IBDV mutant according to the invention is replication competent in cell culture (Example 2). The VP5⁻ IBDV exhibits a delay in replication in chicken embryo cells as compared to the VP5⁺ parental virus, however, final yields of the virus are similar, i.e. about $10^{7.5}$ TCID₅₀/ml (Example 1). Moreover, it is demonstrated that the IBDV mutant is also able to

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infect poultry and to replicate in the infected host animals <u>in vivo</u>, i.e. evidence is provided that the gene encoding the VP5 protein is a non-essential gene. Example 3 shows that the VP5 IBDV can be re-isolated from organs of animals infected with the IBDV mutant and that the IBDV mutant induces a protective immune response in the infected animals.

Morcover, it has been established herein that part of the normal anti-IBDV immune response in poultry is directed to the VP5 region. This is rather surprising as the VP5 protein is considered to represent a non-structural viral protein (Mundt et al., J. Gen. Virol. 76, 437-443, 1995) and the immune response in an animal against a viral pathogen is usually elicited against the structural (glyco)proteins of the virus. These findings make the IBDV mutant and other birnavirus mutants according to the present invention a suitable vaccine candidate for a marker vaccine. Such a marker vaccine provides the possibility to determine whether animals are infected with a wild-type birnavirus, e.g. IBDV, or with a vaccine virus.

Additionally, it has been found that the VP5 protein is involved in the expression of virulence of the birnaviruses, in particular of IBDV, and that the inability of the virus mutants to produce the native VP5 protein leads to an attenuation of the virus.

With the term "which is not able to produce a native VP5 protein" is meant that the birnavirus mutant produces a polypeptide that can be distinguished by serological tests from the native VP5 protein, or does not produce a VP5 protein at all. For example, in the former case, the birnavirus mutant produces only a fragment of the native birnavirus VP5 protein which lacks one or more immunogenic epitopes.

Preferably, the birnavirus mutant according to the invention produces no VP5 protein upon infection of a host cell.

As described above, the genomic organisation of the birnaviruses is well established: the IBDV and IPNV genome comprises a large segment A and a smaller segment B. The segment A of IBDV comprises a large open reading frame (ORF) encoding a polyprotein of about 110 kDa (VP2-VP4-VP3). The gene encoding the VP5 protein is identified in the prior art, and defined herein, as the small ORF on segment A of the birnavirus genome which precedes and partially overlaps the polyprotein encoding ORF (Bayliss et al., J. Gen. Virol. 71, 1303-1312, 1990; Spies et al., J. Gen. Virol. 71, 977-981, 1990; Havarstein L.S. et al., J. Gen. Virology 71, 299-308; 1990; Dobos et al., 1995, supra; Figures 1-3 herein and SEQ ID No.'s 1-7). The mutation introduced in the VP5 gene is such that it does not prevent the expression of the polyprotein.

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SEQ ID No. 1 comprises the full length cDNA nucleotide sequence of segment B of IBDV strain P2, as well as the amino acid sequence of the VP1 protein encoded by segment B (see also SEQ ID. No. 2). SEQ ID No. 3 and 5 depict the full length cDNA sequence of segment A of IBDV strain D78 and the coding region of the VP5 protein and the polyprotein, respectively. SEQ ID 3 and 4 also show the amino acid sequence of the D78 VP5 protein. SEQ ID No. 5 and 6 show the amino acid sequence of the polyprotein VP2-VP4-VP3 of D78. SEQ ID No. 7 shows the 5'-end of segment A of strain D78, including the mutations introduced in the VP5 coding region. SEQ ID No. 8 shows the nucleotide sequence of segment B of strain D78 and the amino acid sequence of the D78 VP1 protein. The genomic organisation of both segments is also shown in Figure 1.

The ORF coding for VP5 is conserved in all hitherto published segment A sequences. The IBDV ORF encodes 145 amino acids resulting in a calculated molecular mass of 16.5 kDa. The nucleotide sequence of the ORF encoding the VP5 protein of IBDV strain D78 used herein is shown in SEQ ID No. 3 and 4. Natural variations may exist between individual IBDV isolates. These natural variations result from small differences in the genomes of these viruses. The nucleotide sequence of the segment A, including the nucleotide sequence of the VP5 gene for many IBDV isolates have been described in the prior art (Vakharia et al., Avian Diseases 36, 736-742, 1992; Bayliss et al., J. Gen. Virol. 71, 1303-1314, 1990; Hudson et al., Nuc. Acid Res. 14, 5001-5012, 1986; Schnitzler et al., J. Gen. Virol. 47, 1563-1571, 1993; Kibenge et al., J. Gen. Virol. 71, 569-577, 1990 and Virology 184, 437-440, 1991; Mundt et al., Virology 209, 10-18, 1995; Lana et al., Virus Genes 6, 247-259, 1992; Vakharia et al., Virus Res. 31, 265-273, 1994; Brown et al., Virus Res. <u>40</u>, 1-15, 1996). The amino acid sequence of the VP5 protein from serotype I IBDV strains display a homology of at least 95% with the VP5 amino acid sequence shown in SEQ ID No. 3 and 4, whereas the homology between serotype II VP5 sequence and the amino acid sequence shown in SEQ ID No. 3 and 4 is at least 75%. Therefore, a preferred IBDV mutant according to the present invention is an IBDV mutant wherein the mutation is introduced in the VP5 gene having a homology of at least 75%, in particular at least 95% on the amino acid sequence level with the VP5 amino acid sequence shown herein.

Preferably an IBDV mutant according to the present invention is derived from any of the classical or variant (e.g. variant E or GLS) IBDV vaccine strains, such as those currently used in the field. Such suitable IBDV strains include the IBDV vaccine strains present in the

commercially available vaccines: D78, PBG 98, LZ 228E, 89-03 (Intervet International B.V.), Bursine 2 (Fort Dodge Animal Health) and S 706 (Rhône Mérieux).

A particular preferred IBDV mutant according to the invention is derived from the D78.

A particular preferred IBDV mutant according to the invention is derived from the D78 strain comprising a VP5 gene encoding a protein having the amino acid sequence shown in SEQ ID No. 3 and 4.

Alternatively, the parent birnavirus strain for the virus mutant according to the invention is a virulent birnavirus field strain. It is found herein that the VP5 protein is a factor associated with virulence, and that the absence of the native VP5 protein in a birnavirus results in an attenuated form of the virus.

Preferably the invention provides a birnavirus mutant which is not able to produce a native VP5 protein as a result of a mutation in the part of the VP5 gene which does not overlap with the large ORF encoding the polyprotein.

In particular, the birnavirus mutant according to the invention comprises a mutation in the 5'-end of the VP5 gene spanning nucleotides 1-30, preferably 1-20, more preferably 1-10. Most preferred is an birnavirus mutant having a mutation in nucleotides 1-3 of the VP5 gene.

A mutation is understood to be a change of the genetic information in the VP5 gene with respect to the genetic information present in this region of the genome of naturally occurring birnavirus producing native VP5 protein. The mutation is, for example, a nucleic acid substitution, deletion, insertion or inversion, or a combination thereof.

In a preferred embodiment of the present invention a birnavirus mutant is provided wherein the mutation is a substitution of one or more nucleotides. In particular, a nucleic acid substitution is introduced in the start codon, as a result of which the new codon encodes an amino acid different from methionine or represents a stop codon, preferably the nucleic acid substitution comprises at least two of the nucleotides of the start codon.

A further birnavirus mutant according to the invention comprises a substitution of one or more nucleotides in a codon(s) different from the start codon resulting in one or more stop codons, preferably in the 5'-end of the VP5 gene as defined above, if desired in addition to a substitution in the start codon as described above. Preferably, the birnavirus mutant comprises a stop codon in this region of the VP5 gene in each of the three reading frames.

Such a preferred birnavirus mutant may be an IBDV mutant having a mutation in the start codon, the fourth and the sixth codon of the VP5 gene, preferably resulting in the mutated codons shown in SEQ ID No. 7 and Figure 3.

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Alternatively, a birnavirus mutant is provided wherein the mutation is a deletion. In particular, the deletion comprises less than 20, less than 10 or less than 5 nucleotides. Preferably, the deletion comprises a total number of nucleotides not dividable by three, resulting in a shift of the reading frame.

Preferably the deletion comprises one or more nucleotides of the start codon of the VP5 gene.

In an alternative embodiment of the present invention a birnavirus mutant is provided wherein the mutation comprises the insertion of a heterologous nucleic acid sequence in the birnavirus genome. A heterologous nucleic acid sequence is a nucleic acid sequence normally not present at the specific insertion site of the particular virus species.

The heterologous nucleic sequence to be incorporated into the birnavirus genome is a nucleic acid fragment which either encodes a polypeptide or is a non-coding sequence. The nucleic acid fragment can be derived from any source, e.g. viral, eukaryotic, prokaryotic or synthetic, including oligonucleotides suitable for the interruption of the expression of the VP5 gene.

A suitable oligonucleotide for the interruption of the VP5 expression may comprise three translational stop codons in each of the possible reading frames in both directions, in addition to one or more appropriate restriction enzyme cleavage sites useful for the insertion of a second heterologous nucleic acid sequence. The length and nucleotide sequence of such a non-coding heterologous nucleic acid sequence is not critical, but preferably varies between 8-50 nucleotides.

In a further embodiment of the present invention a birnavirus mutant is provided which can be used not only for the preparation of a vaccine against infection by a specific birnavirus, but also against other poultry or fish infectious diseases. For example, a vector vaccine based on such an IBDV mutant offers the possibility to immunise against other avian pathogens by the expression of antigens of these avian pathogens within infected cells of the immunised host. Such an IBDV vector according to the present invention can be obtained by inserting a heterologous nucleic acid sequence encoding a polypeptide heterologous to the IBDV in the VP5 gene as defined herein.

The heterologous nucleic acid sequence may encode an antigen of an avian pathogen such as Newcastle disease virus, Infectious bronchitis virus, Marek's disease virus, avian

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Furthermore, an IBDV mutant according to the invention comprises in addition to the mutation in the VP5 gene, a mutation in the VP2 gene, wherein this gene expresses a chimeric protein comprising neutralising epitopes of more than one antigenic type of IBDV (e.g. classic, Variant-E and/or GLS). Preferably, such a mutant comprises the relevant protective VP2 epitopes of a variant GLS strain and classic strain. In particular, the mutated VP2 gene is a GLS VP2 gene comprising a nucleic acid sequence fragment encoding the B69 epitope. The construction of such a mutated VP2 genes is described in Snyder et al., Avian Diseases 38, 701-707, 1994.

Furthermore, nucleic acid sequences encoding polypeptides for pharmaceutical or diagnostic applications, in particular immuno-modulators such as lymphokines, interferons or cytokines, may be incorporated into the VP5 gene. The heterologous nucleic acid sequence may also encode a screenable marker, such as E. coli β -galactosidase or E. coli β -glucuronidase.

The construction of birnavirus mutants, in particular of IBDV mutants according to the present invention can be achieved by means of the recently established infectious cRNA system for IBDV (Mundt and Vakharia, Proc. Natl. Acad. Sci. USA 93, 11131-11136, 1996). This reverse genetics system opens the possibility to introduce mutations in the RNA genome of the IBD virus, in particular in the VP5 gene. The most important step in this reverse genetics system is to provide full length cDNA clones of the segments A and B of IBD virus. cDNA constructs comprising the segment A or B, including the nucleotides of the 5'- and 3'- ends of both these segments can be generated according to the method described by Mundt and Vakharia (1996, supra). Additionally, these constructs comprise a RNA polymerase promoter operably linked to either of the segments. The promoter can be the promoter for the T7, SP6 or T3 polymerase, the T7 promoter being preferred. Mutations can be introduced into the VP5 gene by means of methods generally known in the art for this purpose. In particular, the mutation(s) are introduced by means of site directed mutagenesis.

For example, in a first step a cDNA fragment is provided comprising at least a substantial part of the VP5 gene. In the next step suitable primer pairs are designed and hybridised with the VP5 sequence containing fragment. The 5'-primer comprises in addition to sequences complementary to the VP5 sequence, nucleotides which harbour the desired mutation, e.g. a mutation which changes the ATG start codon to an AGG (arginine) codon. Moreover, the 5'-

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primer is provided with an upstream nucleotide sequence representing a suitable restriction enzyme cleavage site which allows the restoring of the complete 5'-end non-coding sequence. Subsequently, the new mutated fragment is amplified using PCR and the new fragment is introduced in the starting sequence by replacing the native nucleic acid sequence using appropriate restriction enzymes. In the next step plus-sense transcripts of the segment A and B are generated in vitro with (T7) RNA polymerease, after which the synthetic transcripts are purified using conventional RNA purification techniques. The recombinant IBDV mutant according to the invention is obtained after transfection of suitable cells (e.g. VERO cells, QM-7 cells or CEC cells) with the synthetic RNA transcripts of both segments of the IBDV genome, if desired in the presence of transfection-enhancing compositions, such as Lipofectin. Finally the recombinant IBDV is harvested from the supernatant of the transformed cells.

Methods for introducing a mutation in the birnavirus genome are described herein, but are also generally used in the art (Mundt and Vakharia, 1996, supra; Current Protocols in Molecular Biology, eds.: F. M. Ausubel et al., Wiley N.Y., 1995 edition, pages 8.5.1.-8.5.9.)

Further to the unexpected finding by the present inventors that the VP5 ORF of IBDV is a non-essential region of the IBDV genome, it has also been found that an IBDV mutant according to the present invention is able to induce a protective immune response, i.e. animals immunised with a vaccine comprising the IBDV mutant are protected against virulent challenge. Moreover, it has been found that anti-sera of animals infected with naturally occurring IBDV comprise antibodies directed to the non-structural VP5 protein and that these antisera can be distinguished from anti-sera derived from animals infected with an IBDV mutant according to the present invention. In addition, it has been found that the IBDV mutant as described above is attenuated if compared with the parent IBD virus which is able to produce the native VP5 protein.

Therefore, another aspect of this invention is a vaccine for use in the protection of animals against birnavirus infection comprising the birnavirus mutant as characterised above, together with a pharmaceutical acceptable carrier or diluent. In particular, the vaccine according to the invention is a vaccine for use in the protection of poultry against infectious bursal disease comprising the IBDV mutant described above.

The birnavirus mutant according to the present invention can be incorporated into the vaccine as live or inactivated virus.

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A vaccine according to the invention can be prepared by conventional methods such as for example commonly used for the commercially available live- and inactivated IBDV vaccines. Briefly, a susceptible substrate is inoculated with an IBDV mutant according to the invention and propagated until the virus replicated to a desired infectious titre after which IBDV containing material is harvested.

Every substrate which is able to support the replication of IBD viruses can be used in the present invention, including primary (avian) cell cultures, such as chicken embryo fibroblast cells (CEF) or chicken kidney cells (CK), mammalian cell lines such as the VERO cell line or the BGM-70 cell line, or avian cell lines such as QT-35, QM-7 or LMH. Usually, after inoculation of the cells, the virus is propagated for 3-10 days, after which the cell culture supernatant is harvested, and if desired filtered or centrifuged in order to remove cell debris.

Alternatively, the IBDV mutant is propagated in embryonated chicken eggs. In particular, the substrate on which these IBD viruses are propagated are SPF embryonated eggs. Embryonated eggs can be inoculated with, for example 0.2 ml IBDV mutant containing suspension or homogenate comprising at least 10² TCID₅₀ per egg, and subsequently incubated at 37 °C. After about 2-5 days the IBD virus product can be harvested by collecting the embryo's and/or the membranes and/or the allantoic fluid followed by appropriate homogenising of this material. The homogenate can be centrifuged thereafter for 10 min at 2500 x g followed by filtering the supernatant through a filter (100 μm).

The vaccine according to the invention containing the live virus can be prepared and marketed in the form of a suspension or in a lyophilised form and additionally contains a pharmaceutically acceptable carrier or diluent customary used for such compositions. Carriers include stabilisers, preservatives and buffers. Suitable stabilisers are, for example SPGA, carbohydrates (such as sorbitol, mannitol, starch, sucrose, dextran, glutamate or glucose), proteins (such as dried milk serum, albumin or casein) or degradation products thereof. Suitable buffers are for example alkali metal phosphates. Suitable preservatives are thimerosal, merthiolate and gentamicin. Diluents include water, aqueous buffer (such as buffered saline), alcohols and polyols (such as glycerol).

If desired, the live vaccines according to the invention may contain an adjuvant. Examples of suitable compounds and compositions with adjuvant activity are the same as mentioned below.

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Although administration by injection, e.g. intramuscular, subcutaneous of the live vaccine according to the present invention is possible, the vaccine is preferably administered by the inexpensive mass application techniques commonly used for IBDV vaccination. For IBDV vaccination these techniques include drinking water and spray vaccination.

Alternative methods for the administration of the live vaccine include in ovo, eye drop and beak dipping administration.

In another aspect of the present invention a vaccine is provided comprising the birnavirus mutant in an inactivated form. The major advantage of an inactivated vaccine is the extremely high levels of protective antibodies of long duration that can be achieved.

The aim of inactivation of the viruses harvested after the propagation step is to eliminate reproduction of the viruses. In general, this can be achieved by chemical or physical means. Chemical inactivation can be effected by treating the viruses with, for example, enzymes, formaldehyde, β -propiolactone, ethylene-imine or a derivative thereof. If necessary, the inactivating compound is neutralised afterwards. Material inactivated with formaldehyde can, for example, be neutralised with thiosulphate. Physical inactivation can preferably be carried out by subjecting the viruses to energy-rich radiation, such as UV light or γ -rays. If desired, after treatment the pH can be adjusted to a value of about 7.

A vaccine containing the inactivated birnavirus mutant can, for example comprise one or more of the above-mentioned pharmaceutically acceptable carriers or diluents suited for this purpose.

Preferably, an inactivated vaccine according to the invention comprises one or more compounds with adjuvant activity. Suitable compounds or compositions for this purpose include aluminium hydroxide, -phosphate or -oxide, oil-in-water or water-in-oil emulsion based on, for example a mineral oil, such as Bayol F® or Marcol 52® or a vegetable oil such as vitamin E acetate, and saponins.

The vaccine according to the invention comprises an effective dosage of the birnavirus mutant as the active component, i.e. an amount of immunising birnavirus material that will induce immunity in the vaccinated birds against challenge by a virulent virus. Immunity is defined herein as the induction of a significant higher level of protection in a population of birds after vaccination compared to an unvaccinated group.

Typically, the live vaccine according to the invention can be administered in a dose of 10^2-10^9 TCID₅₀ infectious dose₅₀ (TCID₅₀) per animal, preferably in a dose ranging from 10^{50} -

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 10^{70} TCID₅₀, and an inactivated vaccines may contain the antigenic equivalent of 10^5 - 10^9 TCID₅₀ per animal.

Inactivated vaccines are usually administered parenterally, e.g. intramuscularly or subcutaneously.

Although, the IBDV vaccine according to the present invention may be used effectively in chickens, also other poultry such as turkeys, guinea fowl and partridges may be successfully vaccinated with the vaccine. Chickens include broilers, reproduction stock and laying stock.

The age of the animals receiving a live or inactivated vaccine according to the invention is the same as that of the animals receiving the conventional live- or inactivated IBDV vaccines. For example, broilers (free of maternally derived antibodies-MDA) may be vaccinated at one-day-old, whereas broilers with high levels of MDA are preferably vaccinated at 2-3 weeks of age. Laying stock or reproduction stock with low levels of MDA may be vaccinated at 1-10 days of age followed by booster vaccinations with inactivated vaccine on 6-8 and 16-20 weeks of age.

The invention also includes combination vaccines comprising, in addition to the IBDV or IPNV mutant according to the invention, one or more immunogens derived from other pathogens infectious to poultry or fish, respectively.

Preferably, the combination vaccine additionally comprises one or more vaccine strains of infectious bronchitis virus (IBV), Newcastle disease virus (NDV), egg drop syndrome (EDS) virus, turkey rhinotracheitis virus (TRTV) or reovirus.

In addition to a marker vaccine for birnaviruses, the availability of an appropriate diagnostic test is an essential requirement for the application of a birnavirus eradication control programme. Such a diagnostic test is provided herein and comprises a method for determining IBDV infection in poultry and IPNV infection in fish, i.e. it provides a method for distinguishing an animal in the field vaccinated with a vaccine as described above, from an animal infected with a naturally-occurring IBDV or IPNV.

Therefore, the present invention provides a method for the detection of birnavirus infection, in particular for the detection of IBDV infection in an animal comprising the step of examining a sample of the animal for the presence of VP5 antibodies or antigens. The animal is an animal from the field and is in particular an avian species, preferably a chicken. The sample

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A preferred method for determining birnavirus infection in an animal is a method for the detection of antibodics against the VP5 protein, comprising the steps of:

- (i) incubating a sample suspected of containing anti-birnavirus antibodies, with VP5 antigen,
- (ii) allowing the formation of antibody-antigen complex, and
- (ii) detecting the presence of the antibody-antigen complex.

The design of this immunoassay may vary. For example, the immunoassay may be based upon competition or direct reaction. Furthermore, protocols may use solid supports or may use cellular material. The detection of the antibody-antigen complex may involve the use of labelled antibodies; the labels may be, for example, enzymes, fluorescent-, chemiluminescent-, radio-active- or dye molecules.

Suitable methods for the detection of the VP5 antibodies in the sample include the enzyme-linked immunosorbent assay (ELISA), immunofluorescent test (IFT) and Western blot analysis.

In an exemplifying ELISA, the wells of a polystyrene micro-titration plate are coated with VP5 antigen. Next, the wells of the coated plates are filled with chicken serum and serial dilutions are made. After incubation, chicken anti-VP5 protein serum antibodies are determined by detecting antibody (monoclonal or polyclonal) with the same specificity as the coated one, but which is labelled (e.g. with biotin). The labelled antibody will occupy the free antigens that have not been occupied by anti-VP5 antibodies in the chicken serum. For example, horse radish peroxidase coupled to avidin may be added and the amount of peroxidase is measured by an enzymatic reaction. If no antibodies against VP5 are present in the chicken serum sample then a maximum absorption is obtained. If the serum contains many antibodies against VP5 then a low absorption is expected. Alternatively, after the incubation with chicken serum, the amount of antibodies present in the serum that bound to the VP5 antigen may be determined directly by using an anti-chicken conjugate followed by the enzymatic reaction.

In a sandwich ELISA the wells of a polystyrene micro-titration plate can be coated with a monoclonal antibody directed against the VP5 protein. Next, the wells of these coated plates are incubated with VP5 antigen. After the antigen is captured, the wells are filled with the chicken serum and scrial dilutions are made. Subsequently, the protocol as described above may be

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followed. This test can also be carried out by using polyclonal serum against VP5 instead of the coated monoclonal antibodies.

In another diagnostic test (Western blot analysis), the VP5 antigen (containing) material is subjected to SDS-PAGE. Next, the separated proteins are electroblotted onto nitro-cellulose membrane. Thereafter, the membranes can be cut into lanes and the lanes are incubated with the chicken serum. The presence of VP5 antibodies in the sample can be determined by examination whether antibodies bound to the VP5 antigen, for example by using an anti-chicken conjugate followed by an enzymatic reaction. If antibodies against VP5 are present then a band at about 17 kDa is identifiable.

The VP5 antigen may be any VP5 protein (fragment) comprising material which allows the formation of the VP5 antigen-VP5 antibody complex. Preferably, the VP5 antigen comprises the expression product of a conventional recombinant host cell or virus, e.g. such as E.coli expressed VP5 (Mundt et al., J. Gen. Virol. 76, 437-443, 1995) or baculovirus expressed protein (Vakharia et al., Vaccine 12, 452-456, 1994; Vakharia et al., J. Gen Virol. 74, 1201-1206, 1993). In a further embodiment of the present invention a diagnostic test kit is provided which is suitable for performing the diagnostic test according to the invention as described above.

In particular, a diagnostic test kit is provided which comprises in addition to the components usually present, the VP5 antigen (if desired coated onto a solid phase) as the immunological reagent. Other components usually present in such a test kit include, biotin or horseradish peroxidase conjugated antibodies, enzyme substrate, washing buffer etc.

To determine birnavirus VP5 antigen in a test sample from an animal in the field, VP5-specific antibodies are used as the immunological reagent, preferably fixed to a solid phase. The test sample is added, and after an incubation time allowing formation of the antibody-antigen complex, a second labelled antibody may be added to detect the complex.

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EXAMPLES

Example 1.

Construction and analysis of recombinant VP5 IBD virus

Construction of full length VP5 clone of IBDV segment A.

To construct a VP5-negative IBDV, the *Eco*RI site immediately following the 3'-end of the full length cDNA of strain D78 segment A (pUC19FLAD78; Mundt and Vakharia, Proc. Natl. Acad. Sci. USA <u>93</u>, 11131-11136, 1996) was deleted. An *Eco*RI - *Kpn*I fragment containing the T7 polymerase binding site followed by the complete segment A sequence was excised and inserted into *Eco*RI - *Kpn*I cleaved vector pUC18 after inactivation of the unique *Nde*I within the vector sequence resulting in plasmid pAD78/EK. Thereafter, the genomic region encompassing the initiation codon for VP5 was amplified in two pieces using primers A1F5' and VP5MutR, and VP5MutF and A2R, respectively (see Table 1 for sequence and location of primers). PCR fragments were cloned separately and were subsequently fused via a unique *AfI*II site which had been created by mutations within respective primers (see Fig. 2). An *Eco*RI - *Nde*I fragment containing the T7 polymerase binding site, and the 5'-part of segment A including the introduced mutations was excised and used to substitute the wild-type *Eco*RI - *Nde*I fragment in pAD78/EK to yield plasmid pAD78/VP5'. Of the three mutations introduced one altered the initiation methionine codon for VP5 into an arginine codon (Fig. 2).

<u>Table 1</u>: Sequence of oligonucleotide primers used for generating mutant constructs.

^a Nucleotide sequence	Orientation	Designation	Nucleotide no.
AGAGAATTC <i>TAATACGACTCACTATA</i> GGA	+	A1F5`	1-18
TACGATCGGTCTGAC			
TGGGCCTGTCACTGCTGTCACATGT	-	A2R	716 - 740
CATTGCTCTGCAGTGTGTAGTGAGC	-	A3R	338 - 362
CTACAACGCTATCCTTAAGGGTTAGTA	+	VP5MutF	80 - 109
GAG			
<u>CTCTACTAACCCTTAAGGATAGCGTTGT</u>	-	VP5MutR	80 - 109
AG			

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a) Underlined nucleotides denote virus specific nucleotides. T7 promotor sequences are marked in italics. Mutated nucleotides are bold and orientation of the primer is shown for sense (+) and antisense (-). Primer positions are given according to the published sequence of serotype I strain P2 (Mundt et al., Virology 209, 209-218, 1995).

Virus recovery from cRNA. For *in vitro* transcription of RNA plasmids pAD78/EK, pAD78/VP5⁻ and pBP2 (Fig. 2) were linearized by cleavage with *Bsr*GI and *Pst*I, respectively. Treatment of linearized DNA, transcription and purification of RNA, and transfection were carried out as described by Mundt and Vakharia (1996, supra) with the exception that secondary CEC were used for the transfection experiments. Three days after transfection a CPE was visible in CEC. Cells were freeze/thawed, centrifuged at 700 x g to eliminate cellular debris, and the resulting supernatants were filtrated through 0.45 μm filters and stored at -20°C. For the transfection experiments full length cDNA clones of segment A of strain D78 capable of expressing (pAD78/EK) or unable to express VP5 (pAD78/VP5⁻) were transcribed into synthetic RNA and cotransfected with segment B full length cRNA into CEC. Resulting virus progeny IBDV/EK and IBDV/VP5⁻ was further characterised.

Analysis of transfection immunofluorescence progeny by and Radioimmunoprecipitation assay (RIPA). VP5 was expressed in E.coli as described in Mundt et al. (J. Gen. Virol. 76, 437-443, 1995). Rabbit monospecific polyclonal anti serum and mouse monoclonal antibodies against VP5 were prepared according to standard protocols. Vero cells infected with IBDV/VP5, IBDV/EK, and non-infected cells, respectively, were incubated with rabbit anti-IBDV serum, rabbit anti-VP5 serum and with anti-VP5 mAb DIE 7. and stained with fluoresceine-conjugated secondary antibodies. Both antisera and the monoclonal antibody recognised IBDV antigens in the cytoplasm of IBDV/EK infected cells. In contrast, whereas the anti-IBDV serum readily detected viral antigens in IBDV/VP5 infected cells, neither the monospecific anti VP5-serum nor the monoclonal anti-VP5 antibody exhibited specific reactivity. None of these immunological reagents reacted with non-infected controls.

To analyse viral proteins expressed during replication lysates of radioactively labelled CEC infected with IBDV/VP5⁻ (Fig 4, lanes 1-3) and IBDV/EK (Fig. 4, lanes 4-6) were immunoprecipitated with rabbit anti-IBDV serum, rabbit anti-VP5 serum and mAb DIE 7. Non-infected CEC were used as control (Fig. 4, lanes 7-9). IBDV/EK (lane 4) as well as IBDV/VP5⁻ (lane 1) infected CEC showed viral proteins VP2, VP3, and VP4 after precipitation with rabbit anti-IBDV serum. The rabbit anti-VP5 serum (lane 5) and mAb DIE 7 (lane 6) precipitated VP5 with a molecular mass of 21 kDa only from IBDV/EK infected cells. No specific reactivity was detectable in IBDV/VP5⁻ infected CEC after precipitation with rabbit-anti VP5 (lane 2) as well as the VP5 specific mAb DIE 7 (lane 3). Non-infected CEC showed no specific reactivity (lanes 7-9).

Replication of IBDV/VP5⁻ in CEC. To assay replication of IBDV/VP5⁻ in more detail one step growth was analysed (Fig. 5). Confluent secondary CEC were infected with IBDV/EK and IBDV/VP5⁻ with 10⁷² TCID₅₀, respectively. Immediately after overlaying the infected cells with 5 ml growth medium, supernatant from one infected CEC tissue plate of each virus was removed and stored at -20°C (0 h p.i.). Remaining tissue culture plates were further incubated and 4h, 8h, 16h, 24h, and 48h p.i. supernatants were removed and stored at -20°C. Supernatants were centrifuged and titrated according to standard methods. The TCID₅₀ at the different time points after infection showed that the VP5 expressing virus (IBDV/EK) replicated faster than the virus mutant lacking VP5 (IBDV/VP5⁻). 16 h after infection IBDV/EK showed a 100-fold higher than IBDV/VP5⁻ (Fig. 5). However, at 48 h p.i. IBDV/VP5⁻ reached a titre of 10⁷² TCID₅₀/ml which was similar to IBDV/EK (10^{7,45}/ml)

Preparation of recombinant IBDV VP5⁻-2. Plasmid pAD78/VP5⁻-2 was prepared by techniques similar to those described above. The nucleotide sequence of part of the mutated VP5 gene is shown in SEQ ID No. 7 and Figure 3. A restriction enzyme fragment harbouring the mutations was used to substitute the wild-type *EcoRI* - *NdeI* fragment in pAD78/EK. An outline of the protocol for the preparation of the recombinant plasmid is shown in Figure 3. The organisation of pBD78 is also depicted in Figure 3. The recombinant virus was prepared as described above, except for the fact that segment B of strain D78 (SEQ ID No. 8) was used and QM-7 cells were used for the transfection experiment.

Example 2

Identification of VP5 protein in different IBDV strains

Different strains of IBDV were investigated for the expression of the VP5-gene. This was done by making use of the immuno-fluorescence technique (IFT). Chicken embryo fibroblasts grown in microtiterplates were infected with different IBDV strains. Three to 5 days after incubation at 37°C cells were fixed with 70% ethanol, then treated with polyclonal rabbit anti IBDV serum (R1928), polyclonal rabbit anti VP5 serum (RαVP5) or monoclonal antibody directed against VP5 (DIE7), respectively. Binding of the poly- or monoclonal antibodies to the different IBDV strains was visualised by making use of a fluorescence labelled conjugate (goatanti-rabbit or goat-anti-mouse). The results are shown in Table 2:

<u>Table 2</u>: Identification of different sero- and subtypes of IBDV strains. Determination of the presence of VP5 proteins.

IBDV-	IBDV-	IBDV-strain	R1928	RaVP5	DIE7
serotype	subtype				
I	Classical	D78	+	+	+
I	Classical	228TC	+	+	+
I	Classical	PBG98	+	+	+
I	Classical	Ram0404	+	+	+
I	Classical	IBDV/EK	+	+	+
I	Classical	IBDV/VP5	+	-	<u> </u>
I	GLS	GLS	+	+	+
I	Variant-E	8903	+	+	+
II	TY89	TY89	+	+	+

From these data it can be concluded that the different strains of IBDV belonging to different sero- and subtypes do express the VP5-gene. Furthermore, the recombinant VP5-

IBDV vaccine strain can be differentiated from field and vaccine viruses, thereby enabling the recombinant VP5⁻ virus to be used as a marker vaccine.

Example 3

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In vivo testing of the recombinant VP5⁺ and VP5⁻ IBDV vaccines in comparison with a commercial available live IBDV vaccine.

Preparation of IBDV vaccine. Primary chicken embryo fibroblast (CEF) cells were prepared at a final concentration of 2x10°/ml. The cells were cultured in Eagles minimum essential medium containing 5% fetal calf serum. To 25 ml of this cell suspension 0.1 ml IBDV/EK or IBDV/VP5⁻ virus (having an infectious titre of about 3.0 log10 TCID₅₀/ml) was added. After incubation for 5 days in a high-humidity incubator at 37°C, the total suspension was used in the animal experiment without further purification. The infectious titre of the supernantant was 10⁷¹ TCID50/ml.

Animal experiment. In this study the potency of different vaccines (VP5 positive strain IBDV/EK and a VP5 negative strain IBDV/VP5⁻, and the commercial available IBDV vaccine Nobilis strain D78, Intervet International B.V., NL) was investigated. SPF chicks of 3 weeks old were treated as indicated in the treatment schedule.

Treatment Schedule:

Days after		Groups							
vaccination	1	2	3	4					
00	IBDV/EK	IBDV/VP5	D78	-					
03	Х	x1	x	х					
07	x,bl	x1,bl	x,b	x,bl					
14	x,bl	x,bl	x,bl	x,bl					
20	x,bl	x,bl	x,bl	x,bl					
21	ch	ch	ch	ch					
24	Х	х	Х	х					
31	т	+	+	+					

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- VP5⁺ Bursal disease vaccination with VP5 positive vaccine clone, eye-drop route, dose 10⁴⁶ TCID50/animal, 0.1 ml/animal.
- VP5 Bursal disease vaccination with VP5 negative vaccine clone, eye-drop route, dose 10⁵ 9 TCID50/animal, 0.1 ml/animal.
 - D78 Bursal disease vaccination with IBDV VACCINE NOBILIS STRAIN D78, eye-drop route, one field dose.
- ch Challenge with Bursal disease virus, Farragher strain F52/70, eye-drop route, dose 10²⁰ CID50/animal, 0.1 ml/animal.
- 10 bl Serological examination; VN-test and/or Western blotting.
 - x Histological examination (H.E. staining) and MCA-8 ELISA on bursae.
 - x1 Histological examination (H.E. staining) and MCA-8 ELISA on bursae and reisolation of virus from bursa of Fabricius.
 - + Clinical examination and after 10 days histological examination of the bursa.

Detection of virus in the bursa of Fabricius.

Three, 7, 14 and 20 days after eye-drop vaccination, animals were sacrificed and blood and bursae obtained. The presence of virus in the bursa was determined with an enzyme-linked immunosorbent assay (ELISA) making use of the monoclonal antibody 8 (MAB-8). MAB-8 is directed specifically against IBDV. Data are depicted in Table 3.

Furthermore, 3 and 7 days after vaccination, bursae from animals of group 2 were investigated for the presence of the recombinant VP5⁻ virus. For that purpose bursae were homogenised and cultured on chicken embryo fibroblasts. The presence of the VP5⁻ virus was determined by IFT using polyclonal rabbit sera against IBDV or VP5 or monoclonal antibodies against VP5. From 13 out of 15 bursae (87%) investigated, VP5⁻ virus could be reisolated and identified (positive for R1928 and negative for RαVP5 and DIE7). This indicates that the virus upon animal passage is still VP5⁻, indicating that the virus is stable and does not revert to VP5⁻. Furthermore, by using the different poly- and monoclonal antibodies VP5⁻ vaccine virus can be discriminated from all other vaccine and/or field IBDV viruses. Therefore, the VP5⁻ vaccine may be used as a marker vaccine.

Three days after challenge no virus could be detected in groups 1, 2 and 3 with the MCA-8 ELISA. In contrast, all animals of group 4 (non-vaccinated control group) contained challenge

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virus in the bursa of Fabricius, 3 days after challenge. The results show that animals vaccinated with recombinant VP5⁺ (group 1), recombinant VP5⁻ (group 2) and IBDV vaccine Nobilis D78 (group 3) were protected against severe challenge:

5 <u>Table 3:</u> Individual data for detection of virus in the bursa of Fabricius with the MCA-8 ELISA at different days after vaccination or challenge.

	Days after vaccination→			ion→	Days after challenge	
	3	7	14	20	3	
Group↓			Virus d	etection	Protection↓	
1 VP5 ⁺	2/8*	1/7	0/2	0/3	0/5	100%
2 VP5	0/8	0/7	0/2	0/3	0/5	100%
3 D78	1/8	6/7	0/2	0/3	0/5	100%
4 -	0/8	0/7	0/2	0/3	5/5	0%

^{*}Number of positive bursae per total number tested.

Detection of lesions in the bursa of Fabricius.

The microscopic average lesion score induced by the different IBDV (recombinant) vaccines or the challenge virus are depicted in Table 4.

Before challenge, animals vaccinated with the recombinant VP5⁺ IBDV vaccine (group 1) or vaccinated with IBDV vaccine Nobilis D78 (group 3) showed mild to moderate lesions in the bursa. Three days after challenge only chronic lesions were observed in the bursa of Fabricius, indicating that the animals of groups 1 and 3 were protected against challenge. Furthermore, 10 days after challenge only very mild lesions (0-20% lymphocytic depletion) were observed in the bursa of the animals vaccinated with VP5⁺ recombinant IBDV vaccine or with Nobilis vaccine D78. In contrast animals not vaccinated and challenged showed severe lesions 10 days after challenge. In other words all animals (100%) of groups 1 and 3, vaccinated with the VP5⁻ recombinant IBDV vaccine or with Nobilis vaccine D78 were protected against severe challenge.

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Three, 7, 14 and 20 days after vaccination and 3 and 10 days after challenge with the recombinant VP5⁻ IBDV vaccine, animals of group 2 showed no to hardly any lesions (0-20% lymphocytic depletion) in the bursa. All animals of group 2, vaccinated with the VP5⁻ recombinant IBDV vaccine, were protected against severe challenge. When animals vaccinated with the recombinant VP5⁻ IBDV vaccine are compared to animals of groups 1 or 3 (vaccinated with a recombinant VP5⁺ or commercial available vaccine) the recombinant VP5⁻ vaccine induces less lesions and therefore, is safer, milder than the vaccines tested in this experiment.

Three days post-challenge, all non-vaccinated animals of group 4 showed severe acute lesions in the bursa (total lymphocyte depletion, score 5.0). Ten days after challenge, all animals (17 out of 17 animals) showed total lymphocytic depletion, indicating that these animals were not protected against severe challenge. Animals that died after challenge, all showed severe lesions in the bursa of Fabricius. It was concluded that control group 4 was not protected against severe challenge indicating that the test conditions were optimal.

Table 4: Average bursal lesion score at different days after vaccination or challenge. The average lesion score is calculated as follows: all lesion scores from the animals per group on a certain day are added. This number is then divided by the total number of animals investigated in that group on that day. Individual scores range from 1 to 5. Score 0 = no lymphocytic depletion, score 1 = 0 - 20%; score 2 = 20 - 40%; score 3 = 40 - 60%; score 4 = 60 - 80% and score 5 = 80 - 100% lymphocytic depletion (total lymphocytic depletion).

	Days after vaccination→			Days after challenge→			
	3	7	14	20	3	10	
Group↓		Protection↓					
1 VP5 ⁺	0.8	2.9	1.0	1.0	1.0°	0.6	100%
2 VP5	0.0	0.0	0.5	0.0	0.0°	0.1	100%
3 D78	0.1	2.4	3.5	2.0	2.8°	1.1	100%
4 -	0.0	0.0	0.0	0.0	5.0ª	5.0	0%

^a Acute lesions ^c Chronic lesions

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Serological response.

The serological response of the animals was determined by measuring the ability of blood serum to neutralise a classical infectious bursal disease virus strain in a virus neutralising (VN) test. Serum was investigated 3, 7, 14 and 20 days after vaccination. The average neutralising titres are shown in Table 5.

The results show that recombinant IBDV vaccine VP5⁺ applied to chickens of group 1 induced a good and high serological response 20 days after vaccination which is comparable to the serological response of the chickens vaccinated with the commercial IBDV vaccine Nobilis strain D78 (group 3). The recombinant IBDV vaccine VP5⁻ applied to chickens of group 2 induced also a good serological response. A titre of 9.4 log2 was observed 20 days after vaccination. The serological response induced by the recombinant VP5⁻ IBDV vaccine was delayed when compared to the serological response induced by the recombinant IBDV VP5⁺ vaccine or the commercial IBDV vaccine Nobilis strain D78.

The non-vaccinated group 4 showed no serological response to IBDV.

<u>Table 5:</u> Average IBDV-VN-titres for groups 1 to 4 at different days after vaccination, expressed as log2 of the dilution.

Group	Days after vaccination						
	3	7	14	20			
1 VP5 ⁺	$\leq 1.0 \pm 0.0$	7.1 ± 1.7	10.2 ± 1.4	11.9 ± 1.8			
2 VP5	$\leq 1.0 \pm 0.0$	2.1 ± 1.7	6.3 ± 2.9	9.4 ± 1.4			
3 D78	$\leq 1.0 \pm 0.0$	5.2 ± 2.8	10.3 ± 1.3	11.6 ± 1.5			
4 -	$\leq 1.0 \pm 0.0$	$\leq 1.0 \pm 0.0$	$\leq 1.0 \pm 0.0$	$\leq 1.0 \pm 0.0$			

Serological differentiation between antisera.

The scrological response against VP5 was investigated by making use of western blot analysis. For this purpose the VP5 protein was expressed in the E. coli or baculo expression system. The expressed proteins were separated by SDS PAGE. Next the proteins were electroblotted onto a nitro-cellulose membrane. Thereafter, the membrane was cut into lanes

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and the lanes were incubated with rabbit anti-VP5 serum, chicken serum directed against VP5⁺ recombinant vaccine, chicken serum directed against VP5⁻ recombinant vaccine or negative serum from SPF chickens. Data are summarised in Table 6. As can be seen from Table 6, the VP5⁻ serum does not induce a serological response against VP5. In contrast the rabbit anti-VP5 serum and chicken serum directed against VP5⁺ recombinant vaccine do recognise the VP5-protein and thus induces a scrological response against VP5. This indicates that chicken serum may be used to investigate if animals are exposed to a virus that expresses the VP5 protein (e.g. field virus) or to the VP5⁻ recombinant vaccine.

Table 6: Western blot analysis. Serum from animals vaccinated with VP5⁺ or VP5-recombinant vaccine as well as SPF chicken serum and anti VP5-rabbit serum were investigated for their reaction with the VP5-protein.

Identification of serum sample	Immuno-blot
VP5 ⁺ vaccinated animal, serum sample 20d after vaccination	positive
VP5 vaccinated animal, serum sample 20d after vaccination	negative
Non-vaccinated control, serum sample at 20d	negative
Rabbit anti VP5 serum	positive

Mortality and clinical signs.

None of the animals vaccinated with VP5⁺ IBDV vaccine (group 1), vaccinated with recombinant VP5⁻ IBDV vaccine (group 2) or vaccinated with the commercial IBDV vaccine Nobilis strain D78 (group 3), died or showed clinical signs of infectious bursal disease after challenge, indicating that the animals were protected against severe challenge. All animals in the non-vaccinated control group were not protected against severe challenge.

Example 4

In vivo testing of the recombinant VP5 -2 vaccine

Preparation of the IBDV vaccines. Primary chicken embryo fibroblasts (CEF) cells were prepared at a final concentration of 2 x 10⁶/ml. The cells were cultured in Eagles minimum essential medium containing 5% fetal calf serum. To 15 ml of this cell suspension 0.1 ml IBDV/VP5⁻-2 (D78/D78/VP5⁻) virus was added. After incubation for 6 days in a high humidity incubator at 37°C, the supernatant was titrated. The infectious titre of the supernatant was $10^{8.2}$ TCID₅₀/ml. For the second animal experiment the supernatant was diluted to result in a vaccine dose of $10^{5.5}$ TCID₅₀/animal and for the first animal experiment the supernatant was diluted to result in a vaccine dose of $10^{4.0}$ TCID₅₀/animal or $10^{5.0}$ TCID₅₀/egg.

First animal experiment. The effect of the vaccine is assessed by measurement of the serological response and resistance to challenge obtained from administering a challenge virus at the age of 14 days. The vaccine (10^{5.0} TCID₅₀/egg or 10^{4.0} TCID₅₀/animal of D78/D78/VP5⁻) was applied *in ovo* or intramuscularly at day old. Microscopic lesions in the bursa were investigated, 3 and 10 days after challenge. Protection against challenge was determined and the serological response at the age of 14 days old was determined with the VN-test.

1. Average microscopic lesion score in the bursa 3 and 10 days after challenge.

Days post		Group	
challenge	In ovo	Day old	None-vaccinated
3	3.3	0.0	5.0
10	0.2	0.0	5.0

20 2. Protection after challenge

	Group				
	In ovo	Day old	None-vaccinated		
% protection	91.6	100	0		

3. Serological response against IBDV

	Group						
	In ovo	Day old	None-vaccinated				
VN-titre	6.4 ± 1.7	6.4 ± 1.3	<4.0 ± 0.0				

VN-titre is expressed as log2 of the dilution. Animals with a titre <4.0 log2 are considered negative

5 Conclusions

- 1 The D78/D78/VP5⁻ strain is a highly attenuated IBD-virus
- 2 The virus strain is very mild
- 3 The virus can induce a serological response
- 4 The virus can induce protection
- The virus strain can be applied by intramuscular injection to 1 day old SPF chickens and *in ovo* to 18-days-old embryonated SPF-eggs

Second animal experiment. The effect of the vaccine is assessed by measurement of the serological response against IBDV and resistance to challenge obtained from administering a challenge virus, 21 days after administering the Gumboro vaccine. The vaccine (105.5 TCID₅₀/animal of D78/D78/VP5⁻) was applied via the intramuscular route to 14 days old SPF-chickens. Three, 7, 14, and 20 days after vaccination and 3 days after challenge Bursa, spleen, thymus, liver, duodenum, pancreas, ceacal tonsils and harderian gland were investigated for microscopic lesions. Ten days after challenge Bursae were investigated for microscopic lesions. Sera were tested in the VN-test. And mortality was scored after challenge.

1. Percentage mortality after challenge:

	Mortality after challenge
Vaccinated group	0%
Control group	50%

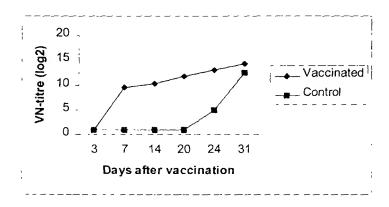
2. Microscopic lesions of the vaccinated group before and after challenge:

Days post	Bursa	Spleen	Thymus	Liver	Duodeum	Pancreas	Ceacal	Harderian
Vaccinat.					†		Tonsils	Gland
3	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0
24	0,A	0	0	0	0	0	0	0
31	0,A	ND	ND	ND	ND	ND	ND	ND

A = None vaccinated animals showed a lymphocytic depletion score of 5.0 (100%) and 4.25,

3 and 10 days after challenge, respectively. ND = not done.

3. Serological response after vaccination:



Conclusions

- 1. The D78/D78/VP5⁻ strain is a highly attenuated IBD-virus
- 10 2. The virus strain is very mild and does not induce lesions in organs
 - 3. The virus can induce a serological response
 - 4. The virus can induce protection

LEGENDS TO THE FIGURES

Figure 1 Genomic organization of segment A and segment B of IBDV. The numbers indicate the nucleotide positions of the start, end and coding region on the segments.

Figure 2 Construction of genomic cDNA clones for the preparation of IBDV/VP5. Plasmid pAD78/EK contains the complete D78 segment A cDNA encoding the polyprotein (VP2-VP4-VP3) and VP5. Plasmid pBP2 contains the complete strain P2 segment B encoding VP1. Mutations were introduced in plasmid pAD78/VP5 altering the methionine start codon for VP5 into arginine and creating an artificial Afl II cleavage site. Recombinant plasmids were linearized with the underlined restriction enzymes, followed by T7 polymerase transcription.

Figure 3 Construction of genomic cDNA clones for the preparation of IBDV/VP5⁻-2. Plasmid pAD78/EK contains the complete D78 segment A cDNA encoding the polyprotein (VP2-VP4-VP3) and VP5. Plasmid pBD78 contains the complete strain D78 segment B encoding VP1. Mutations were introduced in plasmid pAD78/VP5⁻ altering the methionine start codon for VP5 into glutamic acid and creating an artificial BstBI cleavage site. Further mutations were introduced in the arginine and glutamine codon. Recombinant plasmids were linearized with the underlined restriction enzymes, followed by T7 polymerase transcription.

Figure 4 Radioimmunoprecipitation of proteins from CEC infected cells with recombinant IBDV. CEC infected cells with IBDV/VP5 (lanes 1-3), IBDV/EK (lanes 4-6) and uninfected controls were immunoprecipitated with rabbit anti-IBDV serum (lanes 1, 4, 7), rabbit anti-VP5 serum (lanes 2, 5, 8) and mAb DIE 7 (lanes 3, 6, 9). Position of molecular mass markers (M) is indicated. Location of the viral proteins VP2, VP3, VP4 and VP5 are marked.

Figure 5 Replication kinetics of IBDV/EK and IBDV/VP5. Infectious titers of supernatants (vertical axis) are determined at the times indicated.

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SEQUENCE LISTING

45

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5
     (1) GENERAL INFORMATION:
           (i) APPLICANT:
                (A) NAME: Azko Nobel N.V.
                (B) STREET: Velperweg 76
10
                (C) CITY: Arnhem
                (E) COUNTRY: The Netherlands
                (F) POSTAL CODE (ZIP): 6824 BM
                (G) TELEPHONE: 0412 666379
                (H) TELEFAX: 0412 650592
15
         (ii) TITLE OF INVENTION: Recombinant birnavirus vaccine
        (iii) NUMBER OF SEQUENCES: 8
         (iv) COMPUTER READABLE FORM:
               (A) MEDIUM TYPE: Floppy disk
               (B) COMPUTER: IBM PC compatible
               (C) OPERATING SYSTEM: PC-DOS/MS-DOS
               (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)
     (2) INFORMATION FOR SEQ ID NO: 1:
          (i) SEQUENCE CHARACTERISTICS:
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               (A) LENGTH: 2827 base pairs
               (B) TYPE: nucleic acid
               (C) STRANDEDNESS: single
               (D) TOPOLOGY: linear
35
         (ii) MOLECULE TYPE: cDNA
         (ix) FEATURE:
               (A) NAME/KEY: CDS
40
               (B) LOCATION: 112..2745
         (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
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	CCG	CCGCCGCTGG CCGCCACGTT AGTGGCTCCT CTTCTTG								GATG	G ATTCTGCCAC C ATG AGT 11					117	
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5	GAC	ATT	TTC	AAC	AGT	CCA	CAG	GCG	CGA	AGC	ACG	ATC	TCA	GCA	GCG	TTC	165
	Asp	Ile	Phe	Asn	Ser	Pro	Gln	Ala	Arg	Ser	Thr	Ile	Ser	Ala	Ala	Phe	
			5					10					15				
	GGC	АТА	AAG	CCT	ACT	GCT	GGA	CAA	GAC	GTG	GAA	GAA	CTC	TTG	ATC	CCT	213
10			Lys														
	1	20	_,				25					30					
	AAA	GTT	TGG	GTG	CCA	CCT	GAG	GAT	CCG	CTT	GCC	AGC	CCT	AGT	CGA	CTG	261
	Lys	Val	\mathtt{Trp}	Val	Pro	Pro	Glu	Asp	Pro	Leu	Ala	Ser	Pro	Ser	Arg	Leu	
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	CTG	CCC	GAG	AAT	GAG	GAG	TAT	GAG	ACC	GAC	CAA	ATA	CTC	CCA	GAC	TTA	357
A.A.	Leu	Pro	Glu	Asn	Glu	Glu	Tyr	Glu	Thr	Asp	Gln	Ile	Leu	Pro	Asp	Leu	
				70					75					80			
25																	
<i>Z</i> 5			ATG														405
j.j. ⊔ig	Ala	Trp	Met	Arg	Gln	Ile	Glu	_	Ala	Val	Leu	Lys		Thr	Leu	Ser	
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40					133					110					113		
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	Gly	Leu	Lys	Asp	Glu	Val	Thr	Leu	Leu	Thr	Gln	Asn	Ile	Arg	Asp	Lys	
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	ATG Met	AAG Lys 180	Glu	GTC Val	GCC Ala	ACT Thr	GGA Gly 185	Arg	AAC Asn	CCA	A AAC Asn	AAG Lys	Asp	CCI Pro	CTA	A AAG Lys	693
5	CTT Leu 195	Gly	TAC Tyr	ACT Thr	TTT Phe	GAG Glu 200	AGC Ser	ATC	GCG Ala	CAG Gln	CTA Leu 205	Leu	GAC Asp	C ATC	ACA Thr	CTA Leu 210	741
10	CCG Pro	GTA Val	GGC Gly	CCA Pro	CCC Pro 215	GGT Gly	GAG Glu	GAT Asp	GAC Asp	AAG Lys 220	Pro	TGG Trp	GTG Val	CCA Pro	CTC Leu 225	ACA Thr	789
15	AGA Arg	GTG Val	CCG Pro	TCA Ser 230	CGG Arg	ATG Met	TTG Leu	GTG Val	CTG Leu 235	ACG Thr	GGA Gly	GAC Asp	GTA Val	GAT Asp 240	GGC Gly	GAC Asp	837
	TTT Phe	GAG Glu	GTT Val 245	GAA Glu	GAT Asp	TAC Tyr	CTT Leu	CCC Pro 250	AAA Lys	ATC Ile	AAC Asn	CTC Leu	AAG Lys 255	TCA Ser	TCA Ser	AGT Ser	885
	GGA Gly	CTA Leu 260	CCA Pro	TAT Tyr	GTA Val	GGT Gly	CGC Arg 265	ACC Thr	AAA Lys	GGA Gly	GAG Glu	ACA Thr 270	ATT Ile	GGC Gly	GAG Glu	ATG Met	933
25	ATA Ile 275	GCT Ala	ATC Ile	TCA Ser	AAC Asn	CAG Gln 280	TTT Phe	CTC Leu	AGA Arg	GAG Glu	CTA Leu 285	TCA Ser	ACA Thr	CTG Leu	TTG Leu	AAG Lys 290	981
30	CAA Gln	GGT Gly	GCA Ala	GGG Gly	ACA Thr 295	AAG Lys	GGG Gly	TCA Ser	AAC Asn	AAC Lys 300	AAG Lys	AAG Lys	CTA Leu	CTC Leu	AGC Ser 305	ATG Met	1029
35			Asp		TGG Trp												1077
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	TCA Ser	GCT Ala 340	CCA Pro	TCC Ser	CCA Pro	Thr	CAC His	CTC Leu	ATG . Met	ATC Ile	Ser	ATG Met 350	ATC Ile	ACC Thr	TGG Trp	CCC Pro	1173
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			TAC Tyr														1269
			2	1	375				- 5	380				J	385		
5	GAG	TGG	ATA	TTG	GCC	CCG	GAA	GAA	CCC	AAG	GCT	CTT	GTA	TAT	GCG	GAC	1317
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	AAC	ATA	TAC	ATT	GTC	CAC	TCA	AAC	ACG	TGG	TAC	TCA	ATT	GAC	CTA	GAG	1365
10	Asn	Ile	Tyr 405	Ile	Val	His	Ser	Asn 410	Thr	Trp	Tyr	Ser	Ile 415	Asp	Leu	Glu	
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	_	Ile	Leu	Thr	Arg	-	Trp	Ser	Asp	Asn	-	Asp	Pro	Met	Phe		
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aute Entot E - n			TGG								_		_		_	_	1509
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	Asp	Ser	Ser	Cys 470	Leu	Ile	Met	Asn	Leu 475	Gln	Ile	Lys	Thr	Tyr 480	Gly	Gln	
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30	GIY	501	485	ASII	AIG	AIG	1111	490	110	11011	11511	1120	495	200		~	
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			TCC												_		1749
	Glu	Arg	Ser	Ile	Asp 535	Asp	Ile	Arg	Gly	Lys 540	Leu	Arg	Gln	Leu	Val 545	ьеи	
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			ACT Thr 565														1845
5			CTC					CCG					GAA				1893
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10			GCT Ala														1941
15			GGG Gly														1989
20			GTA Val														2037
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25 111 111			GAG Glu														2133
30			GAA Glu														2181
35			CTG Leu														2229
40			AAC Asn														2277
40			TAC Tyr 725														2325
45			AGA Arg														2373

	GAG	AAA	CTC	CAC	AAG	TCC	AAG	CCA	GAC	GAC	CCC	GAT	GCA	GAC	TGG	TTC	2421
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	755					760					765					770	
5	GAA	AGA	TCA	GAA	тОД	CTG	тса	GAC	CTT	ርጥር	GAG	מממ	GCC	GAC	Δ·ΤΤ·C	GCC	2469
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sai 15	AAC	CCA	CAG	ACC	GCC	TCC	AAC	CCC	GTT	GTT	GGG	CTC	CAC	CTG	CCC	GCC	2613
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2 0		820					825					830					
	AAG	AGA	GCC	ACC	GGT	GTC	CAG	GCC	GCT	CTT	CTC	GGA	GCA	GGA	ACG	AGC	2661
	Lys	Arg	Ala	Thr	Gly	Val	Gln	Ala	Ala	Leu	Leu	Gly	Ala	Gly	Thr	Ser	
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25	AGA	CCA	ATG	GGG	ATG	GAG	GCC	CCA	ACA	CGG	TCC	AAG	AAC	GCC	GTG	AAA	2709
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J M	ATG	GCC	AAA	CGG	CGG	CAA	CGC	CAA	AAG	GAG	ACC	CGC	TAAC	CAGCO	CAT		2755
30			Lys														
			-1-	870	J		J		875			,					
	GATO	GGA <i>I</i>	ACC A	ACTCA	AAGA	OA DA	GGAC	ACTAA	A TC	CAGA	ACCC	CGTA	ATCCC	CCG (CCTI	CGCCT	2815
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(2) INFORMATION FOR SEQ ID NO: 2:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 878 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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	Il€	Pro	Lys 35	Val	Trp	Val	Pro	Pro 40	Glu	Asp	Pro	Leu	Ala 45	Ser	Pro	Ser
1	0 Arg	Leu 50	Ala	Lys	Phe	Leu	Arg 55	Glu	Asn	Gly	Tyr	Lys 60	Val	Leu	Gln	Pro
1.	65	Ser	Leu	Pro	Glu	Asn 70	Glu	Glu	Tyr	Glu	Thr 75	Asp	Gln	Ile	Leu	Pro 80
	Asp	Leu	Ala	Trp	Met 85	Arg	Gln	Ile	Glu	Gly 90	Ala	Val	Leu	Lys	Pro 95	Thr
	Leu 0	Ser	Leu	Pro 100	Ile	Gly	Asp	Gln	Glu 105	Tyr	Phe	Pro	Lys	Tyr 110	Tyr	Pro
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	145	Glu	Gly	Leu	Lys	Asp 150	Glu	Val	Thr	Leu	Leu 155	Thr	Gln	Asn	Ile	Arg 160
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	Leu	. Lys	Leu 195	Gly	Tyr	Thr	Phe	Glu 200	Ser	Ile	Ala	Gln	Leu 205	Leu	Asp	Ile
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4	225	Thr	Arg	Val	Pro	Ser 230	Arg	Met	Leu	Val	Leu 235	Thr	Gly	Asp	Val	Asp 240
•		/ Asp	Phe	Glu	Val 245	Glu	Asp	Tyr	Leu	Pro 250	Lys	Ile	Asn	Leu	Lys 255	Ser

	Ser	Ser	Gly	' Leu 260		туг	Val	. Gly	Arg 265		. Lys	Gly	Glu	270		e Gly
5	Glu	Met	: Il∈ 275		Ile	Ser	Asn	Gln 280		. Lei	ı Arg	Glu	Leu 285		Thr	Leu
	Leu	Lys 290		Gly	Ala	Gly	Thr 295		Gly	Ser	Asn	1 Lys	Lys	Lys	Leu	Leu
10	Ser 305		Leu	Ser	Asp	Tyr 310		Tyr	Leu	Ser	Cys 315		Leu	Leu	Phe	Pro 320
15	Lys	Ala	Glu	Arg	Tyr 325	Asp	Lys	Ser	Thr	Trp		Thr	Lys	Thr	Arg	Asn
	Ile	Trp	Ser	Ala 340	Pro	Ser	Pro	Thr	His	Leu	Met	Ile	Ser	Met 350	Ile	Thr
20 20	Trp	Pro	Val 355	Met	Ser	Asn	Ser	Pro 360	Asn	Asn	Val	Leu	Asn 365	Ile	Glu	Gly
	Cys	Pro 370	Ser	Leu	Tyr	Lys	Phe	Asn	Pro	Phe	Arg	Gly 380	Gly	Leu	Asn	Arg
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	Ser	Thr	Leu	Val 500	Leu	Asp	Gln	Trp	Asn 505	Leu	Met	Arg	Gln	Pro 510	Arg	Pro

	Asp	Ser	Glu 515	Glu	Phe	Lys	Ser	Ile 520	Glu	Asp	Lys	Leu	Gly 525	Ile	Asn	Ph∈
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	Leu	Phe	Cys 595	Ser	Ala	Ala	Tyr	Pro 600	Lys	Gly	Val	Glu	Asn 605	Lys	Ser	Leu
20 20	Lys	Ser 610	Lys	Val	Gly	Ile	Glu 615	Gln	Ala	Tyr	Lys	Val 620	Val	Arg	Tyr	Glu
	Ala 625	Leu	Arg	Leu	Val	Gly 630	Gly	Trp	Asn	Tyr	Pro 635	Leu	Leu	Asn	Lys	Ala 640
2 5	Cys	Lys	Asn	Asn	Ala 645	Gly	Ala	Ala	Arg	Arg 650	His	Leu	Glu	Ala	Lys 655	Gly
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	Asn 705	Arg	Pro	Val	Asn	Thr 710	Gly	Gly	Leu	Lys	Ala 715	Val	Ser	Asn	Ala	Leu 720
40	Lys	Thr	Gly	Arg	Tyr 725	Arg	Asn	Glu	Ala	Gly 730	Leu	Ser	Gly	Leu	Val 735	Leu
45	Leu	Ala	Thr	Ala 740	Arg	Ser	Arg	Leu	Gln 745	Asp	Ala	Val	Lys	Ala 750	Lys	Ala
	Glu	Ala	Glu 755	Lys	Leu	His	Lys	Ser	Lys	Pro	Asp	Asp	Pro 765	Asp	Ala	Asp

	Trp	770	Glu	Arg	Ser	Glu	Thr 775	Leu	Ser	Asp	Leu	Leu 780	Glu	Lys	Ala	Asp	
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10	Val	Lys	Asn	Pro 820	Gln	Thr	Ala	Ser	Asn 825	Pro	Val	Val	Gly	Leu 830	His	Leu	
15	Pro	Ala	Lys 835	Arg	Ala	Thr	Gly	Val 840	Gln	Ala	Ala	Leu	Leu 845	Gly	Ala	Gly	
Here is a second	Thr	Ser 850	Arg	Pro	Met	Gly	Met 855	Glu	Ala	Pro	Thr	Arg 860	Ser	Lys	Asn	Ala	
	Val 865	Lys	Met	Ala	Lys	Arg 870	Arg	Gln	Arg	Gln	Lys 875	Glu	Ser	Arg			
255 30			SEQ (A (B (C	UENC) LE) TY , ST) TO	E CH NGTH PE: RAND POLO	SEQ ARAC : 32 nucl EDNE GY:	TERI 61 b eic SS: line	STIC ase acid sing ar	S: pair	s							
35		(ix)	(A) NAI	ME/K	EY: (31									
40		(xi)	SEQ	UENCI	E DE:	SCRII	PTIOI	N: S)	EQ II	D NO	: 3:						
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45	CAGG	ATGG	GA C'	TCCT	CCTT	C TAC	CAAC	GCTA	TCA			GTT A					114

	ACA	AAC	GAT	CGC	AGC	GAT	GAC	AAA	CCT	GCA	AGA	TCA	AAC	CCA	ACA	GAT	162
	Thr	Asn	Asp	Arg	Ser	Asp	Asp	Lys	Pro	Ala	Arg	Ser	Asn	Pro	Thr	Asp	
				10					15					20			
5	man	maa	omm.	G A FIII	7.00	070	ООП	m.cm	C N III	aga	7 7 C	220	aaa	T CC	aaa	ama	27.0
5															GGC Gly		210
	Cys	ser	25	UIS	1111	GIU	PiO	30	мър	Ald	ASII	ASII	35	1111	GIY	Val	
			23					-					3.0				
	CAT	TCC	GGA	CGA	CAC	CCT	GGA	GAA	GCA	CAC	TCT	CAG	GTC	AGA	GAC	CTC	258
10	His	Ser	Gly	Arg	His	Pro	Gly	Glu	Ala	His	Ser	Gln	Val	Arg	Asp	Leu	
		40					45					50					
															TGT		306
15	_	Leu	Gin	Pne	Asp	Cys 60	GIY	GIY	HIS	Arg		Arg	Ala	Asn	Cys		
15	55					80					65					70	
L.	TTT	CCC	TGG	ATT	CCC	TGG	CTC	AAT	TGT	GGG	TGC	TCA	CTA	CAC	ACT	GCA	354
	Phe	Pro	Trp	Ile	Pro	Trp	Leu	Asn	Cys	Gly	Cys	Ser	Leu	His	Thr	Ala	
Ed sen			-		75	_			_	80	_				85		
120 120																	
95 95	GGG	CAA	TGG	GAA	CTA	CAA	GTT	CGA	TCA	GAT	GCT	CCT	GAC	TGC	CCA	GAA	402
15	Gly	Gln	Trp	Glu	Leu	Gln	Val	Arg	Ser	Asp	Ala	Pro	Asp	Cys	Pro	Glu	
* 1				90					95					100			
5 196	a a m	» (()	aaa	CAC	may.	(1) N	CTLA	CTC	CAC	CCT	א פייני	CAC	TCC	CAC	TCT	CAC	450
25															Ser		430
en i Pier	FIO	1111	105	0111	<u> L</u> cu	G1 11	200	110	0111	1114	DCI	O14	115	Olu	501	*****	
(1) -(1)																	
iō	AGT	GAG	GTC	AAG	CAC	ACT	TCC	TGG	TGG	CGT	TT.	TGC	ACT	AAA	CGG	CAC	498
30	Ser	Glu	Val	Lys	His	Thr	Ser	Trp	Trp	Arg	Leu	Cys	Thr	Lys	Arg	His	
		120					125					130					
			~~~	~~~	~.~	CI TO TO	~~~	3.00	220	a.a.m	a* a	man.	oma:		~ » m ~ n	an can	
											GAG		ACTG!	ACA (	3ATGT	TAGCT	551
35	135	гуя	Arg	ALG	Asp	140	PIO	Arg	пув	PIO	145						
33	133					110					113						
	ACA	ATGG	GTT (	GATG:	rctg	CA A	CAGC	CAAC	A TC	AACG	ACAA	AAT	rggg <i>i</i>	AAC (	GTCC:	TAGTAG	611
	GGG	AAGG	GGT (	CACC	GTCC'	IC A	GCTT	ACCC	A CA	rcat.	ATGA	TCT	rggg:	rat (	GTGA	GCTTG	671
40																	
	GTG.	ACCC	CAT '	TCCC	GCAA'	ra Go	GGCT'	rgac(	C CA	'AAAA	TGGT	AGC	CACA'	rgt (	GACA	GCAGTG	731
	አርአ	adec.	CAG	ልረተር	ፐልሮል	מר בי	יים ממד	דמר 4	G CC	ር አጥር:	ΔΤΤΔ	CCA	מייירים	TCA '	TCAC	AGTACC	791
	ACA	المرادر	CAG .	. 2010	. ACA	JU A	11377	LUCA	5 66	J. 3 L G.		CCA		- UF1	LOAC	ACC	191
45	AAC	CAGG	TGG	GGTA	ACAA	TC A	CACT	GTTC'	T CA	GCCA	ACAT	TGA'	TGCC.	ATC .	ACAA	GCCTCA	851
	GCG	TTGG	GGG	AGAG	CTCG	TG T	TTCA	AACA	A GC	GTCC.	ACGG	CCT'	TGTA	CTG	GGCG	CCACCA	911

TCTACCTCAT AGGCTTTGAT GGGACAACGG TAATCACCAG GGCTGTGGCC GCAAACAATG 971 GGCTGACGAC CGGCACCGAC AACCTTATGC CATTCAATCT TGTGATTCCA ACAAACGAGA 1031 5 TAACCCAGCC AATCACATCC ATCAAACTGG AGATAGTGAC CTCCAAAAGT GGTGGTCAGG 1091 CAGGGGATCA GATGTCATGG TCGGCAAGAG GGAGCCTAGC AGTGACGATC CATGGTGGCA 1151 ACTATCCAGG GGCCCTCCGT CCCGTCACGC TAGTGGCCTA CGAAAGAGTG GCAACAGGAT 1211 10 CCGTCGTTAC GGTCGCTGGG GTGAGCAACT TCGAGCTGAT CCCAAATCCT GAACTAGCAA 1271 AGAACCTGGT TACAGAATAC GGCCGATTTG ACCCAGGAGC CATGAACTAC ACAAAATTGA 1331 15 TACTGAGTGA GAGGGACCGT CTTGGCATCA AGACCGTCTG GCCAACAAGG GAGTACACTG 1391 ACTITCGIGA ATACTICATG GAGGIGGCCG ACCICAACTC ICCCCIGAAG ATTGCAGGAG 1451 CATTCGGCTT CAAAGACATA ATCCGGGCCA TAAGGAGGAT AGCTGTGCCG GTGGTCTCCA 1511 CATTGTTCCC ACCTGCCGCT CCCCTAGCCC ATGCAATTGG GGAAGGTGTA GACTACCTGC 1571 TGGGCGATGA GGCACAGGCT GCTTCAGGAA CTGCTCGAGC CGCGTCAGGA AAAGCAAGAG 1631 25 10 4 CTGCCTCAGG CCGCATAAGG CAGCTGACTC TCGCCGCCGA CAAGGGGTAC GAGGTAGTCG 1691 CGAATCTATT CCAGGTGCCC CAGAATCCCG TAGTCGACGG GATTCTTGCT TCACCTGGGG 1751 ũ TACTCCGCGG TGCACACAC CTCGACTGCG TGTT: AGAGA GGGTGCCACG CTATTCCCTG 1811 30 TGGTTATTAC GACAGTGGAA GACGCCATGA CACCCAAAGC ATTGAACAGC AAAATGTTTG 1871 CTGTCATTGA AGGCGTGCGA GAAGACCTCC AACCTCCATC TCAAAGAGGA TCCTTCATAC 1931 35 GAACTCTCTC TGGACACAGA GTCTATGGAT ATGCTCCAGA TGGGGTACTT CCACTGGAGA 1991 CTGGGAGAGA CTACACCGTT GTCCCAATAG ATGATGTCTG GGACGACAGC ATTATGCTGT 2051 CCAAAGATCC CATACCTCCT ATTGTGGGAA ACAGTGGAAA TCTAGCCATA GCTTACATGG 2111 40 ATGTGTTTCG ACCCAAAGTC CCAATCCATG TGGCTATGAC GGGAGCCCTC AATGCTTGTG 2171 GCGAGATTGA GAAAGTAAGC TTTAGAAGCA CCAAGCTCGC CACTGCACAC CGACTTGGCC 2231 45 TTAGGTTGGC TGGTCCCGGA GCATTCGATG TAAACACCGG GCCCAACTGG GCAACGTTCA 2291 TCAAACGTTT CCCTCACAAT CCACGCGACT GGGACAGGCT CCCCTACCTC AACCTACCAT 2351

	ACCTTCCACC	: CAATGCAGGA	CGCCAGTACC	CACCTTGCCAT	GGCTGCATCA	GAGTTCAAAG	2411
	AGACCCCCGA	ACTCGAGAGT	GCCGTCAGAG	CAATGGAAGC	AGCAGCCAAC	GTGGACCCAC	2471
5	TATTCCAATC	TGCACTCAGT	GTGTTCATGT	GGCTGGAAGA	GAATGGGATT	' GTGACTGACA	2531
	TGGCCAACTT	CGCACTCAGC	GACCCGAACG	CCCATCGGAT	GCGAAATTTT	CTTGCAAACG	2591
10	CACCACAAGC	AGGCAGCAAG	TCGCAAAGGG	CCAAGTACGG	GACAGCAGGC	TACGGAGTGG	2651
	AGGCTCGGGG	CCCCACACCA	GAGGAAGCAC	AGAGGGAAAA	AGACACACGG	ATCTCAAAGA	2711
	AGATGGAGAC	CATGGGCATC	TACTTTGCAA	CACCAGAATG	GGTAGCACTC	AATGGGCACC	2771
15	GAGGGCCAAG	CCCCGGCCAG	CTAAAGTACT	GGCAGAACAC	ACGAGAAATA	CCGGACCCAA	2831
	ACGAGGACTA	TCTAGACTAC	GTGCATGCAG	AGAAGAGCCG	GTTGGCATCA	GAAGAACAAA	2891
20	TCCTAAGGGC	AGCTACGTCG	ATCTACGGGG	CTCCAGGACA	GGCAGAGCCA	CCCCAAGCTT	2951
Section of the sectio	TCATAGACGA	AGTTGCCAAA	GTCTATGAAA	TCAACCATGG	ACGTGGCCCA	AACCAAGAAC	3011
#	AGATGAAAGA	TCTGCTCTTG	ACTGCGATGG	AGATGAAGCA	TCGCAATCCC	AGGCGGGCTC	3071
25	TACCAAAGCC	CAAGCCAAAA	CCCAATGCTC	CAACACAGAG	ACCCCCTGGT	CGGCTGGGCC	3131
	GCTGGATCAG	GACCGTCTCT	GATGAGGACC	TTGAGTGAGG	CTCCTGGGAG	TCTCCCGACA	3191
30	CCACCCGCGC	AGGTGTGGAC	ACCAATTCGG	CCTTACAACA	TCCCAAATTG	GATCCGTTCG	3251
	CGGGTCCCCT						3261

35 (2) INFORMATION FOR SEQ ID NO: 4:

40

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 145 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
- 45 Met Val Ser Arg Asp Gln Thr Asn Asp Arg Ser Asp Asp Lys Pro Ala

  1 5 10 15

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Arg Ser Asn Pro Thr Asp Cys Ser Val His Thr Glu Pro Ser Asp Ala
                   20
                                       25
      Asn Asn Arg Thr Gly Val His Ser Gly Arg His Pro Gly Glu Ala His
 5
               35
                                   40
      Ser Gln Val Arg Asp Leu Asp Leu Gln Phe Asp Cys Gly Gly His Arg
           50
                               55
10
     Val Arg Ala Asn Cys Leu Phe Pro Trp Ile Pro Trp Leu Asn Cys Gly
      65
                           70
                                               75
     Cys Ser Leu His Thr Ala Gly Gln Trp Glu Leu Gln Val Arg Ser Asp
                       85
                                           90
15
     Ala Pro Asp Cys Pro Glu Pro Thr Gly Gln Leu Gln Leu Gln Ala
100
                                      105
     Ser Glu Ser Glu Ser His Ser Glu Val Lys His Thr Ser Trp Trp Arg
             115
                                  120
                                                      125
     Leu Cys Thr Lys Arg His His Lys Arg Arg Asp Leu Pro Arg Lys Pro
         130
                              135
                                                  140
25
     Glu
     145
30
     (2) INFORMATION FOR SEQ ID NO: 5:
          (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 3261 base pairs
               (B) TYPE: nucleic acid
35
               (C) STRANDEDNESS: single
               (D) TOPOLOGY: linear
         (ii) MOLECULE TYPE: cDNA
40
         (ix) FEATURE:
               (A) NAME/KEY: CDS
               (B) LOCATION:131..3166
45
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

	CAG	GATG	GGA	CTCC	CTCCI	TC I	'ACAA	CGC	TA TO	CATTO	SATGO	TT#	AGTAC	GAGA	TCAC	ACAAAC	120
5	GAT	CGCA	GCG		ACA Thr												169
10					CTT			Pro					Ala				217
					GAG Glu							Glu					265
15 2 2 2					GGG Gly 50						Leu						313
<b>2</b> 0					TCA Ser					His					Gly		361
25 11 125 11 11					TTC Phe									Asn			409
30					TAC Tyr												457
35					CCT Pro												505
					CAA Gln 130												553
40					TCT Ser												601
45					GAA Glu												649
	GAT	CTT	GGG	TAT	GTG	AGG	CTT	GGT	GAC	CCC	ATT	CCC	GCA	ATA	GGG	CTT	697

	Asp	Let 175	ı Gly	Tyr	· Val	Arg	Leu 180		/ Asp	Pro	) Ile	Pro 185		a Ile	e Gly	/ Leu	
5	GAC Asp 190	Pro	AAA Lys	ATG Met	GTA Val	GCC Ala 195	Thr	TGT Cys	'GAC	: AGC	AGT Ser 200	Asp	AGC Arg	G CCC	AGA Arc	A GTC y Val	745
	TAC Tyr	ACC	: ATA	ACT Thr	GCA Ala	GCC Ala	GAT Asp	'GAT	TAC	CAA	TTC	TCA	TCA	CAG	TAC	CAA	793
10					210					215					220	1	
	CCA Pro	GGT	GGG	GTA Val 225	ACA Thr	ATC Ile	ACA Thr	CTG Leu	TTC Phe 230	TCA Ser	GCC Ala	AAC Asn	ATT	GAT Asp 235	GCC Ala	ATC Ile	841
15 	ACA Thr	AGC Ser	CTC Leu	AGC Ser	GTT Val	GGG Glv	GGA Glv	GAG	CTC	GTG Val	TTT Phe	CAA	ACA	AGC	GTC	CAC	889
			240					245					250				
4 1 20 2 T	GGC Gly	CTT Leu 255	GTA Val	CTG Leu	GGC Gly	GCC Ala	ACC Thr 260	ATC Ile	TAC Tyr	CTC Leu	ATA Ile	GGC Gly 265	TTT Phe	GAT Asp	GGG Gly	ACA Thr	937
25	ACG Thr 270	GTA Val	ATC Ile	ACC Thr	AGG Arg	GCT Ala 275	GTG Val	GCC Ala	GCA Ala	AAC Asn	AAT Asn 280	GGG Gly	CTG Leu	ACG Thr	ACC Thr	GGC Gly 285	985
Stant Paris Mark and Stant Sta	ACC Thr	GAC Asp	AAC Asn	CTT Leu	ATG Met	CCA Pro	TTC Phe	AAT Asn	CTT Leu	GTG Val	ATT Ile	CCA	ACA Thr	AAC	GAG	ATA	1033
30					290					295					300		
35											ATA Ile						1081
	GGT Gly	GGT Gly	CAG Gln 320	GCA Ala	GGG Gly	GAT Asp	CAG Gln	ATG Met 325	TCA Ser	TGG Trp	TCG Ser	GCA Ala	AGA Arg 330	GGG Gly	AGC Ser	CTA Leu	1129
40	GCA Ala	GTG Val 335	ACG Thr	ATC Ile	CAT His	Gly	GGC Gly 340	AAC Asn	TAT Tyr	CCA Pro	GGG Gly	GCC Ala 345	CTC Leu	CGT Arg	CCC Pro	GTC Val	1177
45	ACG Thr 350	CTA Leu	GTG Val	GCC Ala	TAC Tyr	GAA Glu 355	AGA Arg	GTG Val	GCA Ala	ACA Thr	GGA Gly 360	TCC Ser	GTC Val	GTT Val	ACG Thr	GTC Val 365	1225
	GCT	GGG	GTG	AGC	AAC	TTC	GAG	CTG	ATC	CCA	TAA	CCT	GAA	CTA	GCA	AAG	1273

	Ala	Gly	Val	Ser	Asn 370	Phe	Glu	Leu	Ile	Pro 375	Asn	Pro	Glu	Leu	Ala 380	Lys		
5						TAC Tyr												1321
10						AGT Ser												1369
15						TAC Tyr												1417
						CCC Pro 435											-	1465
20						ATA Ile											-	1513
25 11						GCT Ala											1	L561
10 30						GAT Asp											1	1609
35						GCA Ala											1	.657
						AAG Lys 515											1	.705
40						GTA Val											1	753
45						AAC Asn											1	1801
	CTA	TTC	CCT	GTG	GTT	ATT	ACG	ACA	GTG	GAA	GAC	GCC	ATG	ACA	CCC	AAA	1	L849

	Let	ı Ph∈	Pro 560		. Val	Ile	. Thr	Thr 565		. Glu	ı Asp	o Ala	Met 570		Pro	Lys	
5																GAC	1897
3	Ala	575		. ser	. ràs	мет	580		. Val	. Ile	e Glu	ι Gl  γ 585		. Arg	g Glu	Asp	
																GGA	1945
10	590		Pro	Pro	ser	595	Arg	GIY	Ser	Phe	600		, Thr	Leu	Ser	Gly 605	
			GTC														1993
	His	Arg	Val	Tyr	Gly 610	Tyr	Ala	Pro	Asp	Gly 615		Leu	Pro	Leu	Glu 620	Thr	
15																	
I.			GAC Asp														2041
1 4 1 1 2 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1	•	J	•	625				110	630	пор	лър	vai	пр	635	Asp	ser	
20			CTG														2089
ş	116	мес	Leu 640	ser	rys	Asp	Pro	11e 645	Pro	Pro	Ile	Val	Gly 650	Asn	Ser	Gly	
<u>.</u>			GCC														2137
25	Asn	Leu 655	Ala	Ile	Ala	Tyr	Met 660	Asp	Val	Phe	Arg	Pro 665	Lys	Val	Pro	Ile	
			GCT														2185
30	His 670	Val	Ala	Met	Thr	Gly 675	Ala	Leu	Asn	Ala	Cys 680	Gly	Glu	Ile	Glu	_	
																685	
			TTT Phe														2233
35				9	690		2,5	БСИ	AIU	695	Ala	nis	Arg	цец	700	Leu	
33	AGG	TTG	GCT	GGT	CCC	GGA	GCA	TTC	GAT	GTA	AAC	ACC	GGG	CCC	AAC	TGG	2281
			Ala														
40	GCA	ACG	TTC	ATC	AAA	CGT	TTC	ССТ	CAC	ТАА	CCA	CGC	GAC	TCC	GAC	NGC	2220
			Phe 720														2329
	CTC	CCC	TAC	ርጥሮ	ΔΔC	ርጥል	CCA	ጥእሮ	CTT	CCA	CCC	חית ת	COA	CC 3	aaa	ar a	
45			Tyr														2377
		735					740					745					
	TAC	CAC	CTT	GCC	ATG	GCT	GCA	TCA	GAG	TTC	AAA	GAG	ACC	ccc	GAA	CTC	2425

	Tyr 750	His	Leu	Ala	Met	Ala 755	Ala	Ser	Glu	Phe	Lys 760	Glu	Thr	Pro	Glu	Leu 765	
5			GCC														2473
3	Giu	ser	Ala	val	770	Ala	мес	GIU	Ala	775	Ala	Asn	val	Asp	780	Leu	
			TCT Ser														2521
10				785					790					795			
			GAC Asp 800														2569
15	ATG	CGA	AAT	TTT	CTT	GCA	AAC		CCA	CAA	GCA	GGC		AAG	TCG	CAA	2617
			Asn														
<b>2</b> 9			AAG Lys														2665
2"			GAG Glu														2713
25	1111	110	Olu	Olu	850	GIII	Arg	Giu	БуБ	855	1111	Arg	116	261	860	пур	
① ① 30			ACC Thr														2761
			CAC														2809
35	ASII	GIY	His 880	Arg	GIY	PIO	sei	885	GIY	GIII	ьеп	ьуѕ	890	rrp	GIII	ASN	
			GAA Glu												-		2857
40			AAG Lys														2905
45			ATC Ile														2953
	ATA	GAC	GAA	GTT	GCC	AAA	GTC	TAT	GAA	ATC	AAC	CAT	GGA	CGT	GGC	CCA	3001

	Ile	Asp	Glu	Val 945	Ala	Lys	Val	Tyr	Glu 950	Ile	Asn	His	Gly	Arg 955	_	Pro	
5											ACT Thr						3049
10											CCC Pro						3097
15											GGC Gly 1000	Arg					3145
Cash Barth Barth Barth						Leu		TGAC	GCT	CCT (	GGGA	FTCT(	cc ca	GACA	CCAC	e	3196
20	CCC		GTG 1	rggao	CACC	AA TI	rcgg(	CCTTA	A CA	ACAT	CCCA	AAT	rgga:	rcc (	GTTC	ECGGGT	3256
25 miles of the second of the	(2)		(i) S (A (E	SEQUE	ENCE ENGTH PE:	SEQ CHAR H: 10 amin	RACTE 012 a no ac	ERIST aminc	CICS:								
35			MOL	ECUL	E TY	OGY: (PE: ESCRI	prot	ein	SEQ I	D NO	): 6:						
	Met 1	Thr	Asn	Leu	Gln 5	Asp	Gln	Thr	Gln	Gln 10	Ile	Val	Pro	Phe	Ile 15	Arg	
40	Ser	Leu	Leu	Met 20	Pro	Thr	Thr	Gly	Pro 25	Ala	Ser	Ile	Pro	Asp 30	Asp	Thr	
	Leu	Glu	Lys 35	His	Thr	Leu	Arg	Ser 40	Glu	Thr	Ser	Thr	Tyr 45	Asn	Leu	Thr	
45	Val	Gly 50	Asp	Thr	Gly	Ser	Gly 55	Leu	Ile	Val	Phe	Phe 60	Pro	Gly	Phe	Pro	
	Glv	Ser	Tle	Va l	Glv	Δla	Hig	Tyr	Thr	T.e11	Gln	Glv	Δen	Glv	Nan	Tur	

	6	5				70	O				7	5				8	0
5	Ly	s Ph	ne As	sp Gl	n Me 8		ı Lev	ı Thi	r Ala	a Gli 90		n Le	u Pr	o Al	a Se 9		r
	As	n Ty	r Cy	s Are		u Va]	. Ser	Arc	g Ser 105		u Thi	r Val	l Ar	g Sei 11(		r Thi	r
10	Lei	u Pr	0 Gl 11	y Gly 5	y Val	l Tyr	Ala	Leu 120		ı Gly	/ Thi	: Ile	e Ası 125		ı Val	l Thi	?
	Ph€	e Gl 13	n Gl	y Ser	. Lei	ı Ser	Glu 135	Leu	Thr	Asp	) Val	. Ser		Asn	Gly	⁄ Leu	l
15	Met 145	Se:	r Ala	a Thr	Ala	Asn 150	Ile	Asn	Asp	Lys	Ile 155		' Asn	Val	Leu	Val	
20	Gly	Gl:	ı Gl}	/ Val	Thr 165		Leu	Ser	Leu	Pro 170	Thr	Ser	Tyr	Asp	Leu 175		
	Tyr	Va]	l Arg	180	Gly	Asp	Pro	Ile	Pro 185	Ala	Ile	Gly	Leu	Asp 190	Pro	Lys	
2 <b>5</b>	Met	Val	. Ala 195	Thr	Cys	Asp	Ser	Ser 200	Asp	Arg	Pro	Arg	Val 205	Tyr	Thr	Ile	
25 mg grad 100 mg 100 m	Thr	Ala 210	Ala	Asp	Asp	Tyr	Gln 215	Phe	Ser	Ser	Gln	Tyr 220	Gln	Pro	Gly	Gly	
30	Val 225	Thr	Ile	Thr	Leu	Phe 230	Ser .	Ala	Asn	Ile	Asp 235	Ala	Ile	Thr	Ser	Leu 240	
35	Ser	Val	Gly	Gly	Glu 245	Leu	Val :	Phe		Thr 250	Ser	Val	His		Leu 255	Val	
	Leu	Gly	Ala	Thr 260	Ile	Tyr	Leu :		Gly : 265	Phe .	Asp	Gly		Thr 270	Val	Ile	
10	Thr	Arg	Ala 275	Val	Ala	Ala .		Asn (	Gly 1	Leu '	Thr		Gly 285	Thr ,	Asp	Asn	
	Leu	Met 290	Pro	Phe	Asn		Val 1 295	[le ]	Pro S	Thr i		Glu 300	Ile	Thr (	Gln	Pro	
15	Ile 305	Thr	Ser	Ile		Leu ( 310	Glu 1	lle V	Val '		Ser :	Lys	Ser (	Gly (		Gln 320	
	Ala	Glv	Asp	Gln	Met	Ser '	rrn G	er 7	N 1 ~ 7	N ~~ (	~1	<b>0</b>					

					325					330					335	
5	Ile	His	Gly	Gly 340	Asn	Tyr	Pro	Gly	Ala 345		Arg	Pro	Val	Thr 350		Val
	Ala	Tyr	Glu 355	Arg	Val	Ala	Thr	Gly 360	Ser	Val	Val	Thr	Val 365	Ala	Gly	Val
10	Ser	Asn 370	Phe	Glu	Leu	Ile	Pro 375	Asn	Pro	Glu	Leu	Ala 380	Lys	Asn	Leu	Val
	Thr 385	Glu	Tyr	Gly	Arg	Phe 390	Asp	Pro	Gly	Ala	Met 395	Asn	Tyr	Thr	Lys	Leu 400
15	Ile	Leu	Ser	Glu	Arg 405	Asp	Arg	Leu	Gly	Ile 410	Lys	Thr	Val	Trp	Pro 415	Thr
19 19 20	Arg	Glu	Tyr	Thr 420	Asp	Phe	Arg	Glu	Tyr 425	Phe	Met	Glu	Val	Ala 430	Asp	Leu
Section 1	Asn	Ser	Pro 435	Leu	Lys	Ile	Ala	Gly 440	Ala	Phe	Gly	Phe	Lys 445	Asp	Ile	Ile
48 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Arg	Ala 450	Ile	Arg	Arg	Ile	Ala 455	Val	Pro	Val	Val	Ser 460	Thr	Leu	Phe	Pro
Section Sectio	Pro 465	Ala	Ala	Pro	Leu	Ala 470	His	Ala	Ile	Gly	Glu 475	Gly	Val	Asp	Tyr	Leu 480
30	Leu	Gly	Asp	Glu	Ala 485	Gln	Ala	Ala	Ser	Gly 490	Thr	Ala	Arg	Ala	Ala 495	Ser
35	Gly	Lys	Ala	Arg 500	Ala	Ala	Ser	Gly	Arg 505	Ile	Arg	Gln	Leu	Thr 510	Leu	Ala
	Ala	Asp	Lys 515	Gly	Tyr	Glu	Val	Val 520	Ala	Asn	Leu	Phe	Gln 525	Val	Pro	Gln
40	Asn	Pro 530	Val	Val	Asp	Gly	Ile 535	Leu	Ala	Ser	Pro	Gly 540	Val	Leu	Arg	Gly
	Ala 545	His	Asn	Leu	Asp	Cys 550	Val	Leu	Arg	Glu	Gly 555	Ala	Thr	Leu	Phe	Pro 560
45	Val	Val	Ile	Thr	Thr 565	Val	Glu	Asp	Ala	Met 570	Thr	Pro	Lys	Ala	Leu 575	Asn

Ser Lys Met Phe Ala Val Ile Glu Gly Val Arg Glu Asp Leu Gln Pro

				580	)				585	5				590	)	
5	Pro	Ser	Gln 595		g Gly	Ser	Phe	: Ile		J Thr	. Leu	Ser	Gl _y		arg	val
	Tyr	Gly 610		Ala	. Pro	Asp	Gly 615		Leu	Pro	) Leu	Glu 620		Gly	/ Arg	Asp
10	Tyr 625	Thr	Val	Val	Pro	Ile 630	Asp	Asp	Val	Trp	635		Ser	Ile	. Met	Leu 640
	Ser	Lys	Asp	Pro	Ile 645	Pro	Pro	Ile	Val	Gly 650	Asn	Ser	Gly	Asn	Leu 655	Ala
15	Ile	Ala	Tyr	Met 660	Asp	Val	Phe	Arg	Pro 665	Lys	Val	Pro	Ile	His 670	Val	Ala
20	Met	Thr	Gly 675	Ala	Leu	Asn	Ala	Cys 680	Gly	Glu	Ile	Glu	Lys 685	Val	Ser	Phe
<b>20</b>	Arg	Ser 690	Thr	Lys	Leu	Ala	Thr 695	Ala	His	Arg	Leu	Gly 700	Leu	Arg	Leu	Ala
	Gly 705	Pro	Gly	Ala	Phe	Asp 710	Val	Asn	Thr	Gly	Pro 715	Asn	Trp	Ala	Thr	Phe 720
	Ile	Lys	Arg	Phe	Pro 725	His	Asn	Pro	Arg	Asp 730	Trp	Asp	Arg	Leu	Pro 735	Tyr
30	Leu	Asn	Leu	Pro 740	Tyr	Leu	Pro	Pro	Asn 745	Ala	Gly	Arg	Gln	Tyr 750	His	Leu
35	Ala	Met	Ala 755	Ala	Ser	Glu	Phe	Lys <b>7</b> 60	Glu	Thr	Pro	Glu	Leu 765	Glu	Ser	Ala
	Val	Arg 770	Ala	Met	Glu	Ala	Ala 775	Ala	Asn	Val	Asp	Pro 780	Leu	Phe	Gln	Ser
40	Ala 785	Leu	Ser	Val	Phe	Met 790	Trp	Leu	Glu	Glu	Asn 795	Gly	Ile	Val	Thr	Asp 800
	Met	Ala	Asn	Phe	Ala 805	Leu	Ser	Asp	Pro	Asn 810	Ala	His	Arg	Met	Arg 815	Asn
45	Phe	Leu	Ala	Asn 820	Ala	Pro	Gln	Ala	Gly 825	Ser	Lys	Ser	Gln	Arg 830	Ala	Lys
	Tyr	Gly	Thr	Ala	Gly	Tyr	Gly	Val	Glu	Ala	Arg	Gly	Pro	Thr	Pro	Glu

			835					840					845	i		
5	Glu	Ala 850	Gln	Arg	(Glu	Lys	Asp 855		Arg	Ile	: Ser	Lys 860		Met	Glu	Thr
	Met 865	Gly	Ile	Tyr	Phe	Ala 870	Thr	Pro	Glu	Trp	Val 875	Ala	Leu	. Asn	Gly	His 880
10	Arg	Gly	Pro	Ser	Pro 885	Gly	Gln	Leu	Lys	Tyr 890		Gln	Asn	Thr	Arg 895	Glu
	Ile	Pro	Asp	Pro 900	Asn	Glu	Asp	Tyr	Leu 905	Asp	Tyr	Val	His	Ala 910	Glu	Lys
15	Ser	Arg	Leu 915	Ala	Ser	Glu	Glu	Gln 920	Ile	Leu	Arg	Ala	Ala 925	Thr	Ser	Ile
20	Tyr	Gly 930	Ala	Pro	Gly	Gln	Ala 935	Glu	Pro	Pro	Gln	Ala 940	Phe	Ile	Asp	Glu
	Val 945	Ala	Lys	Val	Tyr	Glu 950	Ile	Asn	His	Gly	Arg 955	Gly	Pro	Asn	Gln	Glu 960
	Gln	Met	Lys	Asp	Leu 965	Leu	Leu	Thr	Ala	Met 970	Glu	Met	Lys	His	Arg 975	Asn
And the second s	Pro	Arg	Arg	Ala 980	Leu	Pro	Lys	Pro	Lys 985	Pro	Lys	Pro	Asn	Ala 990	Pro	Thr
30	Gln	Arg	Pro 995	Pro	Gly	Arg	Leu	Gly 1000		Trp	Ile		Thr 1005		Ser	Asp
35	Glu	Asp 1010		Glu												
	(2)	INFO	RMAT	ION	FOR	SEQ	ID N	0: 7	:							
40		(i)	(A (B (C	) LE ) TY ) ST	E CH NGTH PE: RAND	: 32 nucl EDNE	61 b eic SS:	ase acid sing	pair	s						

(ii) MOLECULE TYPE: cDNA

	(ix) FEATURE:	
	(A) NAME/KEY: CDS	
	(B) LOCATION:97531	
5		
J	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:	
	GGATACGATC GGTCTGACCC CGGGGGAGTC ACCCGGGGAC AGGCCGTCAA GGCCTTGTTC	60
10	CAGGATGGGA CTCCTCCTTC TACAACGCTA TCATTC GAA GTT AGT TGA GAT CTG Glu Val Ser * Asp Leu 1 5	114
	ACA AAC GAT CGC AGC GAT GAC AAA CCT GCA AGA TCA AAC CCA ACA GAT Thr Asn Asp Arg Ser Asp Asp Lys Pro Ala Arg Ser Asn Pro Thr Asp  10 15 20	162
20 11	(2) INFORMATION FOR SEQ ID NO: 8:	
<b>2</b> 5	<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 2827 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> (ii) MOLECULE TYPE: cDNA	
<b>3</b> 0		
	<pre>(ix) FEATURE:     (A) NAME/KEY: CDS     (B) LOCATION:1122745</pre>	
35	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:	
	GGATACGATG GGTCTGACCC TCTGGGAGTC ACGAATTAAC GTGGCTACTA GGGGCGATAC	60
40	CCGCCGCTGG CTGCCACGTT AGTGGCTCCT CTTCTTGATG ATTCTGCCAC C ATG AGT  Met Ser  1	117
45	GAC ATT TTC AAC AGT CCA CAG GCG CGA AGC ACG ATC TCA GCA GCG TTC Asp Ile Phe Asn Ser Pro Gln Ala Arg Ser Thr Ile Ser Ala Ala Phe  5 10 15	165
	GGC ATA AAG CCT ACT GCT GGA CAA GAC GTG GAA GAA CTC TTG ATC CCT	213

	Gly	Ile 20	Lys	Pro	Thr	Ala	Gly 25	Gln	Asp	Val	Glu	Glu 30		Leu	. Ile	Pro		
5		Val														CTG Leu 50	:	261
10																TCT Ser	:	309
15		CCC Pro															Ş	357
T. S.		TGG Trp															4	105
<b>20</b>		CCT Pro 100															4	53
2.5 2.5 3.0 3.0 3.0 3.0 3.0 3.0 3.0 3.0 3.0 3.0		CCT Pro															5	01
30		AAG Lys															5	49
35		CTA Leu															5	97
33		TAT Tyr															6	45
40		AAG Lys 180															6	93
45		GGG Gly															7	41
	CCG	GTA	GGC	CCA	ccc	GGT	GAG	GAT	GAC	AAG	CCC	TGG	GTG	CCA	CTC	ACA	7	89

	Pro	Val	Gly	Pro	Pro 215	Gly	Glu	Asp	Asp	Lys 220	Pro	Trp	Val	Pro	Leu 225			
5						ATG Met											837	
10						TAC Tyr											885	
15						GGT Gly											933	
1						CAG Gln 280											981	
20						AAG Lys											1029	
<b>25</b>						TAC Tyr											1077	
30						AGT Ser											1125	
35						ACA Thr											1173	
						CCA Pro 360											1221	
40						AAC Asn											1269	
45						CCG Pro											1317	
	AAC	ATA	TAC	ATT	GTC	CAC	TCA	AAC	ACG	TGG	TAC	TCA	ATT	GAC	CTA	GAG	1365	

	Asn	Ile	Tyr 405		Val	His	Ser	Asn 410	Thr	Trp	Tyr	Ser	Ile 415		Leu	Glu	
5			Glu			TGC Cys							Ala			TAC Tyr	1413
10						GGG Gly 440											1461
1.5						TTT Phe											1509
15 45 45 45						ATA Ile											1557
20						GCC Ala											1605
25						TGG Trp											1653
10 10 30						ATT Ile 520											1701
35						GAT Asp											1749
						TAC Tyr											1797
40						CTT Leu											1845
45						TAT Tyr											1893
	TGT	TCT	GCT	GCG	TAT	CCC	AAG	GGA	GTA	GAG	AAC	AAG	AGT	CTC	AAG	TCC	1941

	Суs 595	s Ser	Ala	a Ala	Tyr	600		Gly	/ Val	l Glu	As:		s Sei	: Lei	ı Lys	s Ser 610	
5	AAA Lys	A GTC	GGG Gly	ATC	GAG Glu 615	Glr	GCA Ala	TAC	C AAC	GTA Val	. Val	C AGO L Arg	G TAT	GAC	G GCC Ala 625	G TTG	1989
10	AGG Arg	TTG	GTA Val	GGT Gly 630	GGT Gly	TGG Trp	AAC Asn	TAC	CCA Pro	Leu	CTC	G AAC 1 Asr	C AAA 1 Lys	GCC Ala 640	Cys	AAG Lys	2037
15	AAT Asn	AAC Asn	GCA Ala 645	GGC Gly	GCC Ala	GCT Ala	CGG Arg	CGG Arg 650	CAT	CTG Leu	GAG Glu	GCC Ala	AAG Lys 655	Gly	TTC Phe	CCA Pro	2085
20 20	CTC Leu	GAC Asp 660	GAG Glu	TTC Phe	CTA Leu	GCC Ala	GAG Glu 665	TGG Trp	TCT Ser	GAG Glu	CTG Leu	TCA Ser 670	Glu	TTC Phe	GGT Gly	GAG Glu	2133
20 20 20	GCC Ala 675	TTC Phe	GAA Glu	GGC Gly	TTC Phe	AAT Asn 680	ATC Ile	AAG Lys	CTG Leu	ACC Thr	GTA Val 685	ACA Thr	TCT Ser	GAG Glu	AGC Ser	CTA Leu 690	2181
25	GCC Ala	GAA Glu	CTG Leu	AAC Asn	AAG Lys 695	CCA Pro	GTA Val	CCC Pro	CCC Pro	AAG Lys 700	CCC Pro	CCA Pro	AAT Asn	GTC Val	AAC Asn 705	AGA Arg	2229
30	CCA Pro	GTC Val	AAC Asn	ACT Thr 710	GGG Gly	GGA Gly	CTC Leu	AAG Lys	GCA Ala 715	GTC Val	AGC Scr	AAC Asn	GCC Ala	CTC Leu 720	AAG Lys	ACC Thr	2277
35	GGT Gly	CGG Arg	TAC Tyr 725	AGG Arg	AAC Asn	GAA Glu	GCC Ala	GGA Gly 730	CTG Leu	AGT Ser	GGT Gly	CTC Leu	GTC Val 735	CTT Leu	CTA Leu	GCC Ala	2325
	ACA Thr	GCA Ala 740	AGA Arg	AGC Ser	CGT Arg	CTG Leu	CAA Gln 745	GAT Asp	GCA Ala	GTT Val	AAG Lys	GCC Ala 750	AAG Lys	GCA Ala	GAA Glu	GCC Ala	2373
40	GAG Glu 755	AAA Lys	CTC Leu	CAC His	Lys	TCC Ser 760	AAG Lys	CCA Pro	GAC Asp	GAC Asp	CCC Pro 765	GAT Asp	GCA Ala	GAC Asp	TGG Trp	TTC Phe 770	2421
45	GAA Glu			Glu													2469
	AGC	AAG	GTC	GCC	CAC	TCA	GCA	CTC	GTG	GAA	ACA	AGC	GAC	GCC	CTT	GAA	2517

2565
2613
2661
2709
2755
2815 2827

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## **CLAIMS**

- 1 A birnavirus mutant which is not able to produce a native VP5 protein as a result of a mutation in the VP5 gene of the birnavirus genome.
- 2 A birnavirus mutant according to claim 1, characterised in that the mutation is a substitution.
- 3 A birnavirus mutant according to claim 1, characterised in that the mutation is an insertion of a heterologous nucleic acid sequence.
- 4 A birnavirus mutant according to claim 3, characterised in that the heterologous nucleic acid sequence encodes a polypeptide and the heterologous nucleic acid sequence is under the control of an expression control sequence regulating the expression of the sequence in a cell infected with the virus mutant.
- 5 A birnavirus mutant according to claims 1-4, characterised in that the birnavirus is infectious bursal disease virus (IBDV).
- 6 A birnavirus mutant according to claim 5, characterised in that the mutation is in the genome of a virulent field virus.
- A birnavirus mutant according to claim 5, characterised in that the mutation is in the genome of vaccine strain, preferably in vaccine strain D78.
- A birnavirus mutant according to claims 5-7, characterised in that the mutant has a mutated start codon and three stop codons in the 5'-end of the VP5 gene as shown in SEQ ID No: 7.
- 9 A birnavirus according to claims 5-8, characterised in that the IBDV expresses a chimeric VP2 protein comprising virus neutralising epitopes of different antigenic IBDV types.

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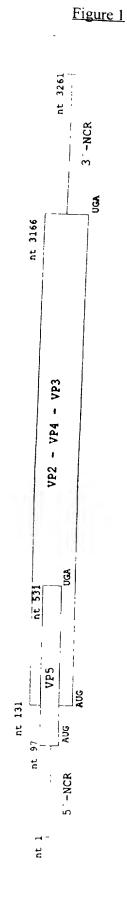
- 10 A vaccine against a birnavirus infection in animals, characterised in that it comprises a birnavirus mutant according to claims 1-9 and a pharmaceutically acceptable carrier.
- 11 A method for determining birnavirus infection in an animal, characterised in that a sample of the animal is examined for the presence of anti-VP5 antibodies.
  - 12 A method according to claim 11, characterised in that the method comprises the steps of:
    - (i) incubating a sample suspected of containing anti-birnavirus antibodies, with VP5 antigen,
    - (ii) allowing the formation of antibody-antigen complex, and
    - (ii) detecting the presence of the antibody-antigen complex.
  - 13 A diagnostic test kit suitable for carrying out a method according to claims 11-12.
- 14 Use of the lack of the expression of native VP5 protein by a birnavirus mutant as a marker to distinguish vaccinated animals from animals infected with naturally-ocurring birnavirus.

## **ABSTRACT**

The present invention provides a birnavirus mutant which is suited as vaccine candidate in eradication control programmes. The mutant is not able to produce a native VP5 protein, and this feature can be used as a marker to distinguish between animals vaccinated with the VP5 mutant or infected with a naturally-occurring birnavirus.

Genomic organization of segment A of strain D78 and segment B of strain P2

D78 segment A



P2 segment B

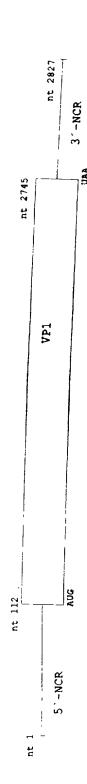
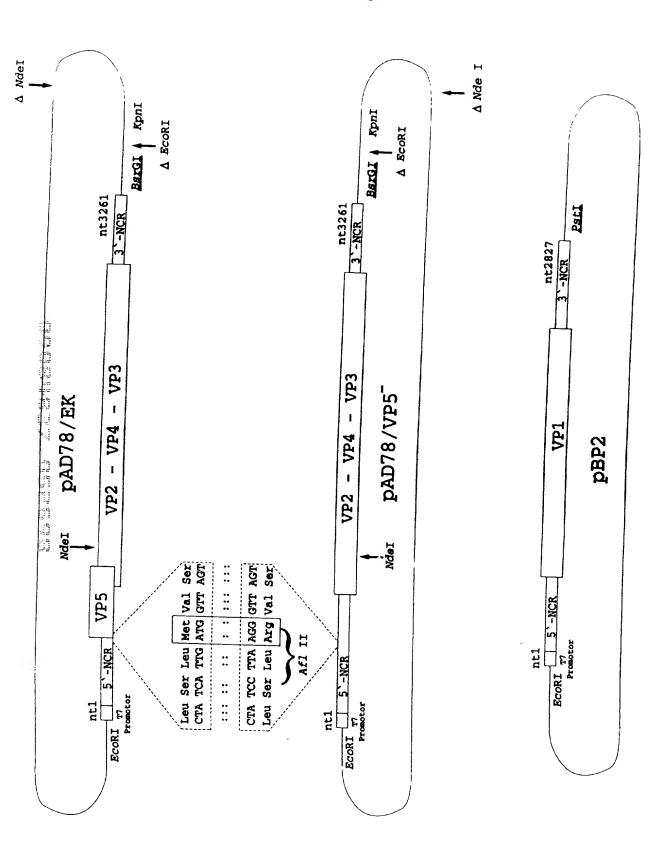


Figure 2



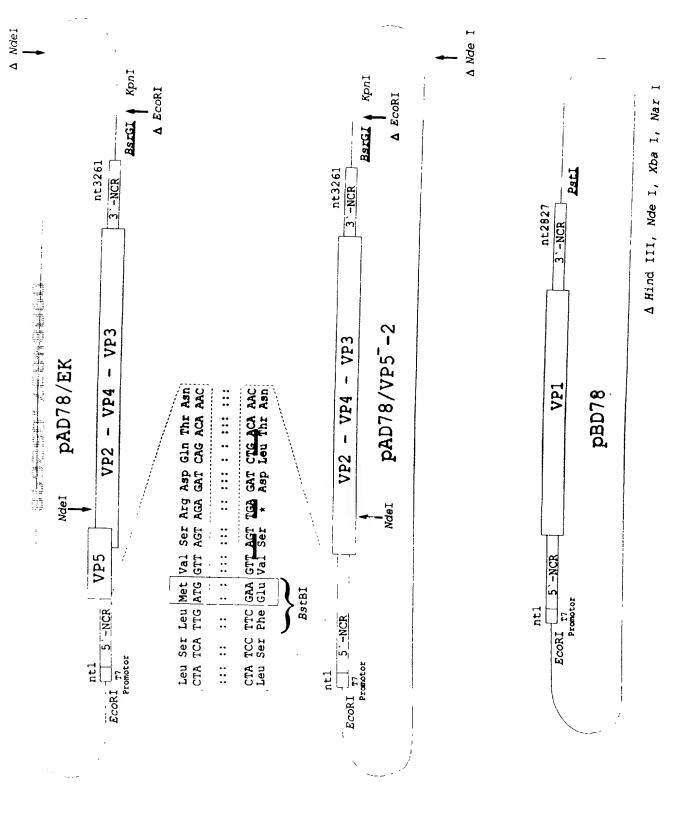
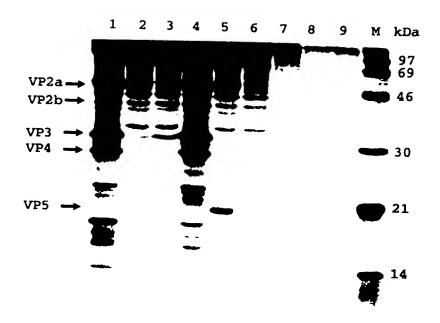


Figure 4



TCID₅₀/ml

Figure 5

